# Chapter 4
# Results & Discussion

## 4.1 Overview of the Results

- In methodology section, we presented work flow, as it shows step by step screening process from low grade to high grade and cancer at the end, results are presented in a same format. The Figure 4.1 depicts the overview of the results.

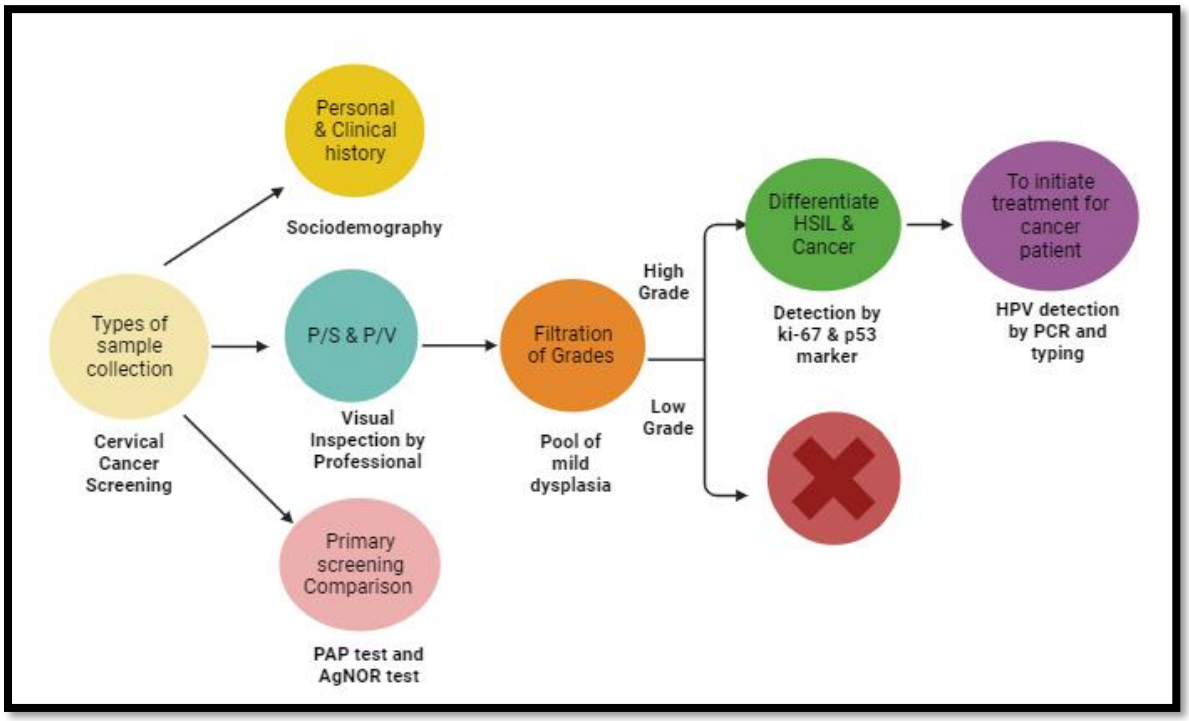


Figure 4.1: Overview of results

## 4.2 Types of specimens collected

- We found out of 498 samples, 86% samples were of cervical and endocervical cancers. The percentage of others types of sample collection, like cervical, cervical and endocervical, vaginal and others (which includes endo and ectocervical) are shown in the chart 4.2.

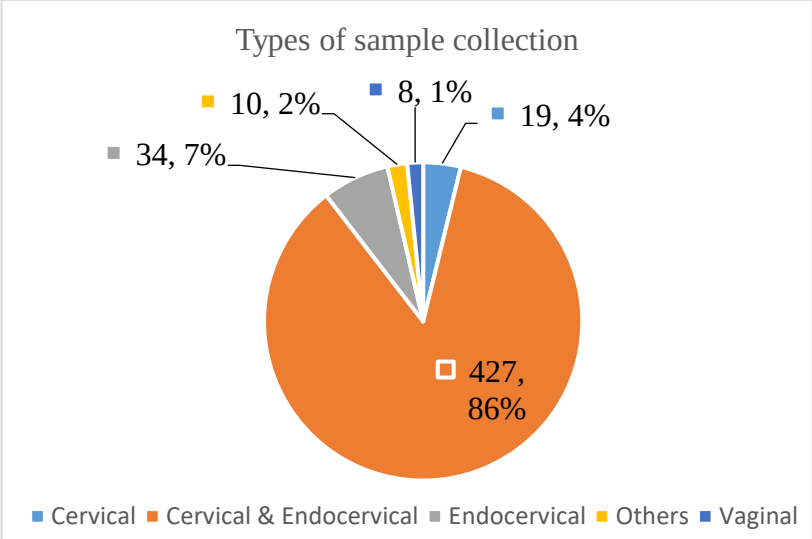


Figure 4.2: Types of samples collected

## 4.3 Sociodemography on the basis of history

### 4.3.1 Personal data

| Personal Data | Age | Frequency | Percentage |
|---|---|---|---|
| Age at the time of screening (n=498) | 18-25 | 50 | 10.0 |
| | 26-35 | 174 | 34.9 |
| | 36-45 | 165 | 33.1 |
| | 46-55 | 66 | 13.3 |
| | >55 | 43 | 8.6 |
| Age of marriage(n=498) | <18 | 246 | 49.4 |
| | 18 | 32 | 6.4 |
| | >18 | 207 | 41.6 |
| | not known | 13 | 2.6 |
| Place of residence (n=498) | Rural | 18 | 3.6 |
| | Urban | 480 | 96.4 |

| | | | |
|---|---|---|---|
| Community (n=498) | Hindu | 449 | 90.2 |
| | Muslim | 48 | 9.6 |
| | Parsi | 1 | 0.2 |
| Occupation(n=498) | Housewife | 487 | 97.8 |
| | Job | 11 | 2.2 |
| Active Sexual Life (n=498) | <1 | 9 | 1.8 |
| | >40 | 12 | 2.4 |
| | 1-10 | 116 | 23.3 |
| | 11-20 | 190 | 38.2 |
| | 21-40 | 171 | 34.3 |

Table 4.1: Personal data of the screening program

- Table 4.1 showing personal data of age, age of marriage, place of resident, community and occupation at the time of screening (n=498).

### 4.3.2 Age at the time of screening

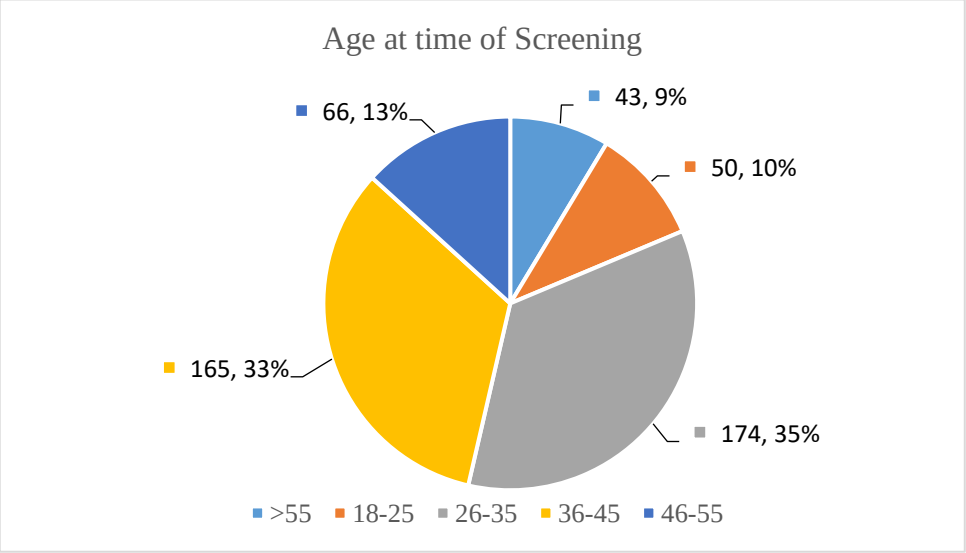


Figure 4.3: Age at the time of screening years of age

- At the time of screening, 10% were of between the ages of 18 to 25 years, 35% are of 26-35 years, 33% are between 36 to 45 years and 13 % are of between the 46-55 years and 9% of females were of above 55

### 4.3.3 Age of marriage

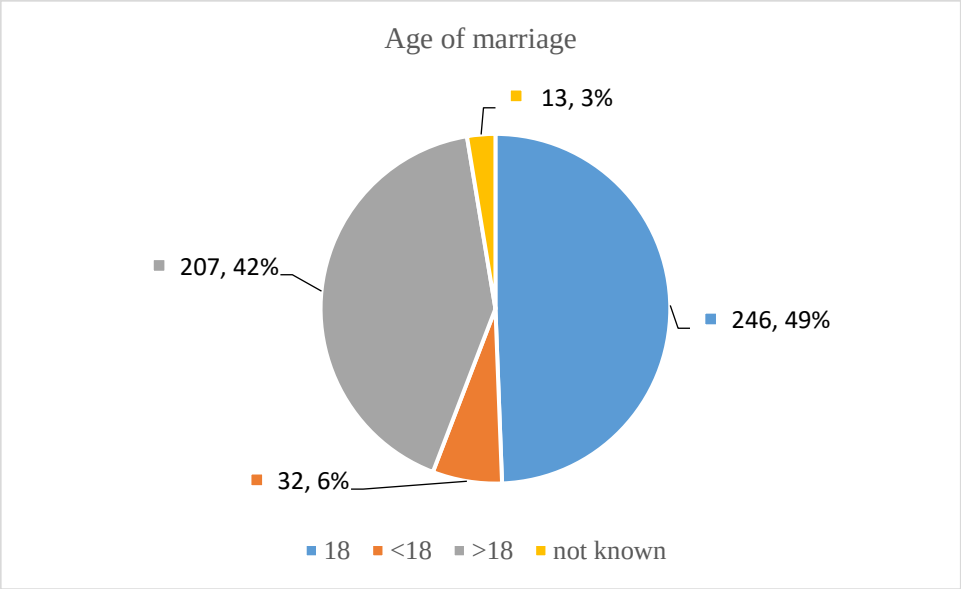


Figure 4.4: Age of Marriage

- At the time of screening, 49% women were married at 18 years of age, 6% of the women were married below 18 years and 42% were married at the age of more than 18% and 3% women were not known their age of marriage. (Fig 4.4)

### 4.3.4 Place of residence

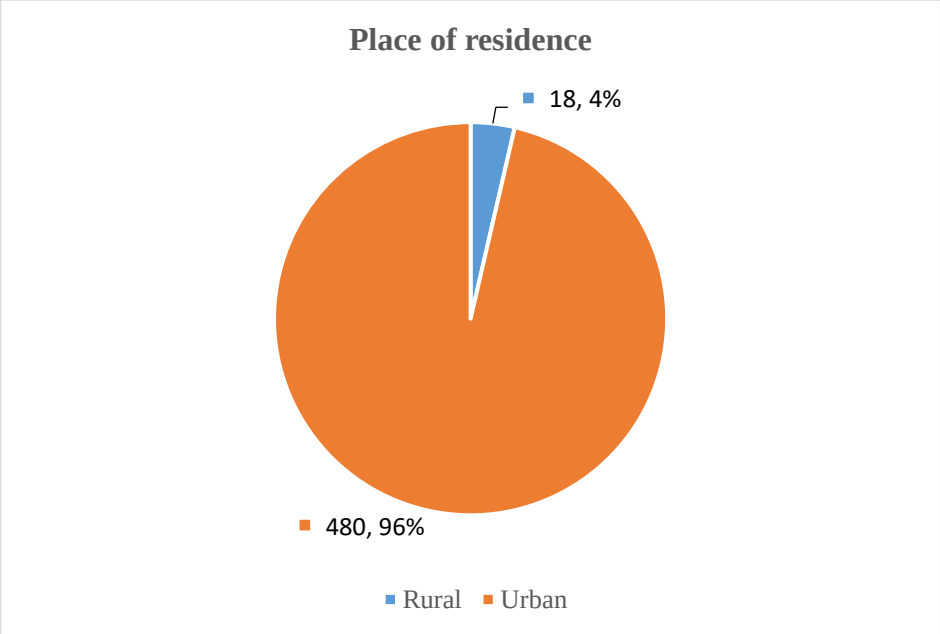


Figure 4.5: Geographic area of females at the time of screening

- At the time of screening geographical distribution showing Fig 4.5. i.e., 96% women were from Urban area and 4 % were from rural area.

### 4.3.5 Community distribution

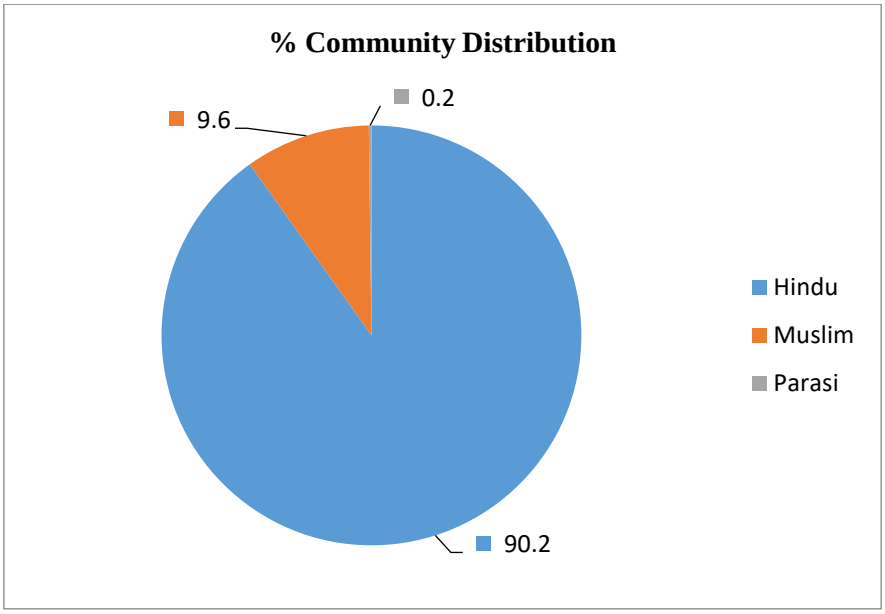


Figure 4.6: Community distribution

- At the time of screening 90.2% women were of Hindu community, 9.6% are of Muslim community and 0.2% are of Parasi community. (Fig 4.6)

### 4.3.6 Occupation

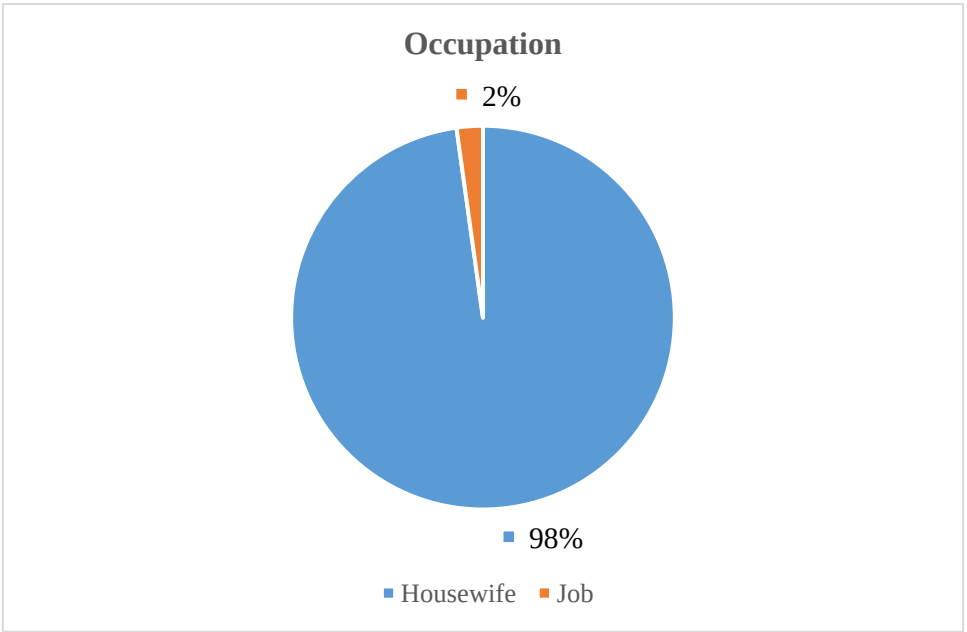


Figure 4.7: Occupation of the females at the time of screening

- At the time of screening 98% females were house wife and 2% female were working women (Fig 4.7)

### 4.3.7 Active sexual life

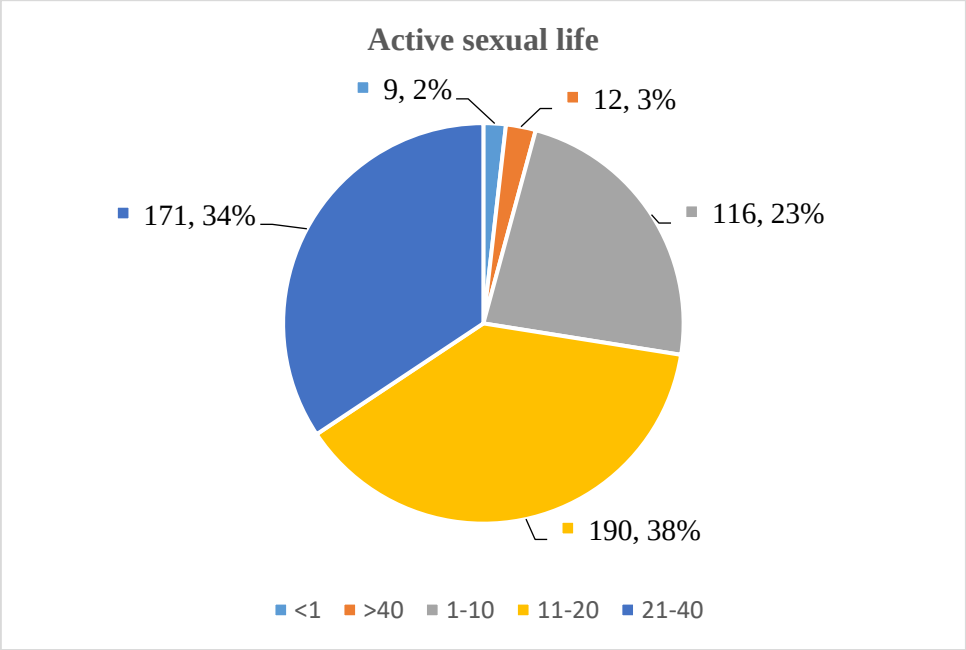


Figure 4.8: Active sexual life

- It was found that 2% women have active sexual life below one year, 23% women have active sexual life between 1-10 Years, 38% women have active sexual life between 11-20 years, 34% women have active sexual life between 21-40 years and 3% woman have active sexual life above 40 years. (Fig 4.8)

### 4.3.8 Menstrual History

- Menstrual history for 498 patients is presented in table 4.2.

| Menstrual history | Age | Frequency | Percentage |
|---|---|---|---|
| Menarche(n=498) | 12 | 3 | 0.6 |
| | 13 | 397 | 79.7 |
| | 14 | 61 | 12.2 |
| | 15 | 20 | 4 |
| | 16 | 7 | 1.4 |
| | 17 | 4 | 0.8 |
| | 18 | 3 | 0.6 |
| | >18 | 3 | 0.6 |

Table 4.2: Menstrual history of screening program

#### 4.3.8.1 Menarche

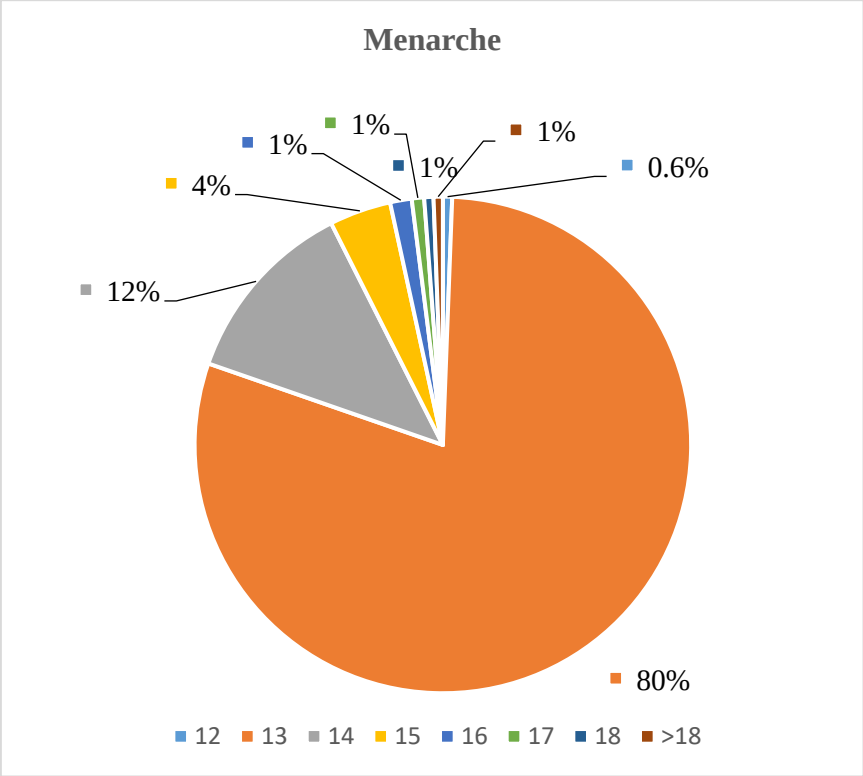


Figure 4.9: Menarche of females at the time of screening

- Approximately 80% females have menarche at the age of 13. While other menarche age was shown in Figure 4.9.

#### 4.3.8.2 Menopause

| Category | Frequency | Percentage |
|---|---|---|
| Menopause | 108 | 25% |
| No Menopause | 323 | 75% |

Table 4.3: Menopause

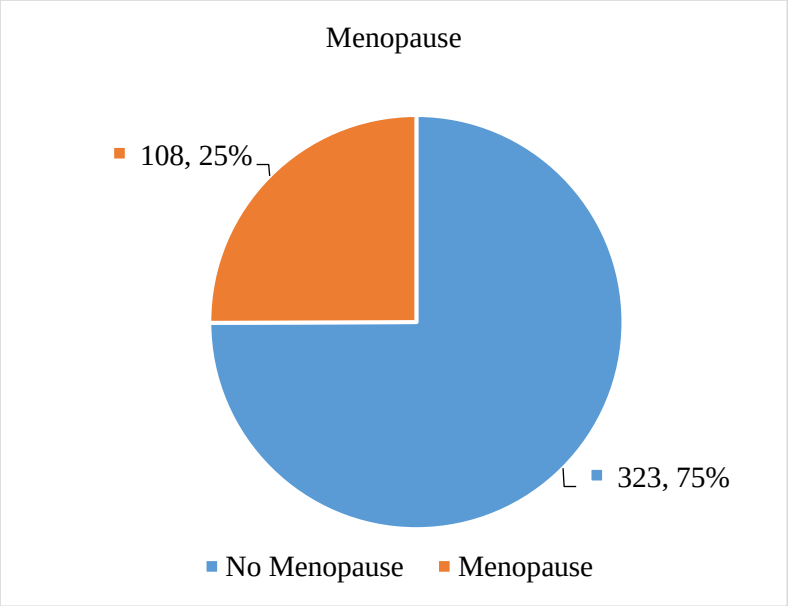


Figure 4.10: Menopause

- At the time of screening, 75% women were not menopausal and 25% female were menopausal women.

### 4.3.9 Labour History

| Labour History | Count | Frequency | Percentage |
|---|---|---|---|
| Mode of delivery (n=447) | FTLSCS | 36 | 8.1 |
| | FTCS | 19 | 4.3 |
| | FTND/FTCS | 5 | 1.1 |
| | FTVD | 295 | 66 |
| | FTVD/FTCS | 7 | 1.6 |
| | FTVD/FTLSCS | 11 | 2.5 |
| | FTND | 74 | 16.6 |
| Place of delivery (n=446) | Home | 31 | 7 |
| | Hospital | 415 | 93 |

Table 4.4: Labor history

- Table 4.4 shows labour history which depicts the data for mode of delivery and their frequency along with place of delivery

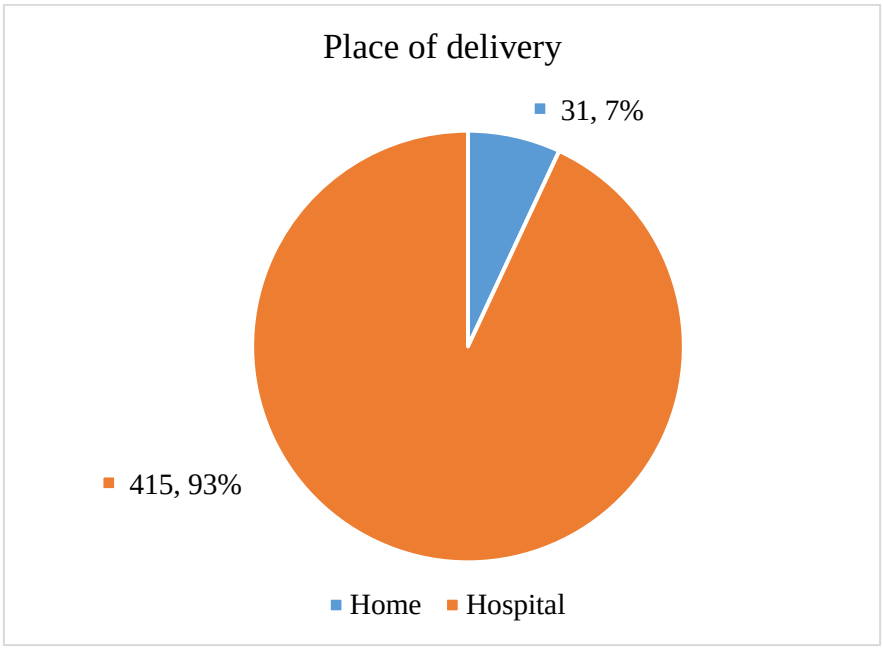


Figure 4.11: Place of Delivery

- 93% females had delivery at hospital while 7% females had delivery at home (Figure 4.11)

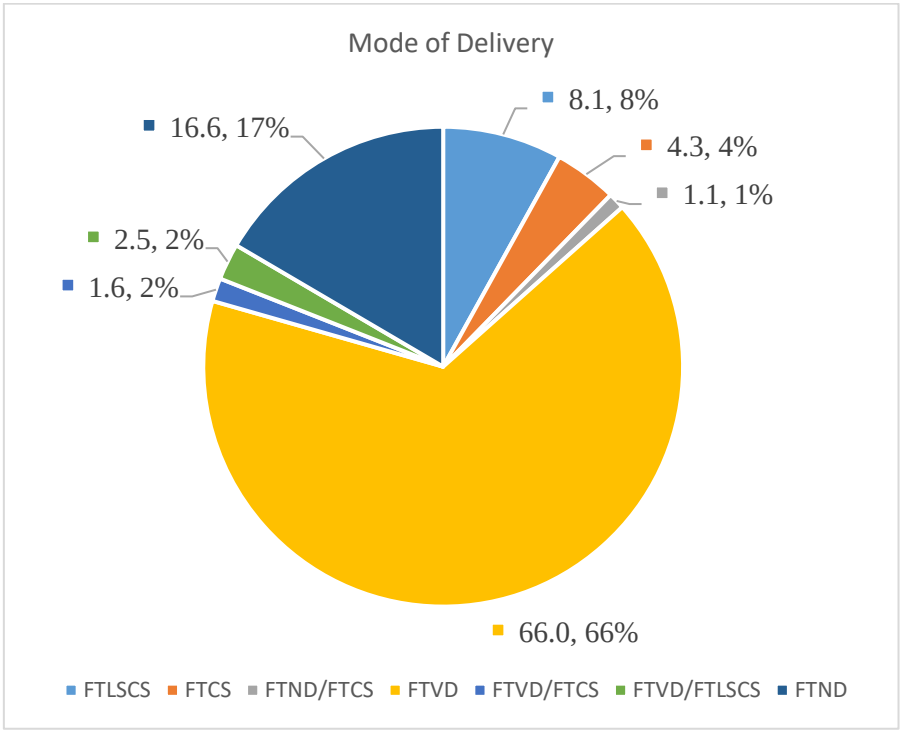


Figure 4.12: Mode of Delivery

- Data shows that majority of mode of delivery was FTVD followed by FTND and FTLSCS.

### 4.3.10 Obstetric History

| Obstetric History | Count | Frequency | Percentage |
|---|---|---|---|
| Gravidity (n=458) | <1 | 4 | 0.9 |
| | 1-5 | 414 | 90.4 |
| | 5-8 | 38 | 8.3 |
| | >8 | 2 | 0.4 |
| Parity (n=457) | 0 | 27 | 5.9 |
| | 1-3 | 383 | 83.8 |
| | 4-6 | 47 | 10.3 |
| Living (n=457) | <1 | 19 | 4.2 |
| | 1-2 | 287 | 62.8 |
| | 3-4 | 133 | 29.1 |
| | >4 | 18 | 3.9 |
| Abortion (n=454) | 0 | 340 | 74.9 |
| | 1-2 | 105 | 23.1 |
| | 3-7 | 340 | 74.9 |
| Tubal ligation (n=440) | No | 386 | 87.7 |
| | Yes | 54 | 12.3 |

Table 4.5: showing Obstetric History

#### 4.3.10.1 Gravidity

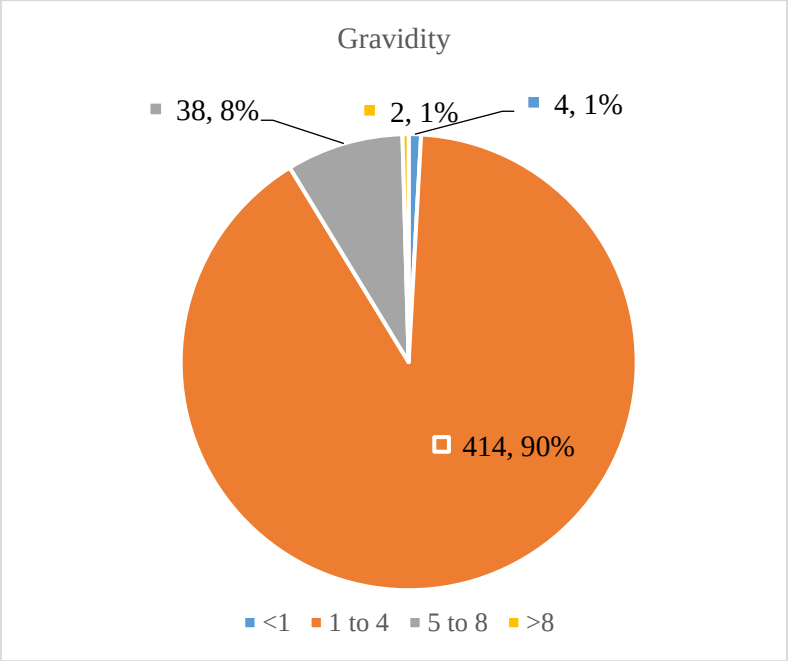


Figure 4.13: Gravidity showing from obstetrics history Maximum

- 90% of are between from 1 to 4. Frequency of patients are 414. (Fig 4.14).

#### 4.3.10.2 Parity

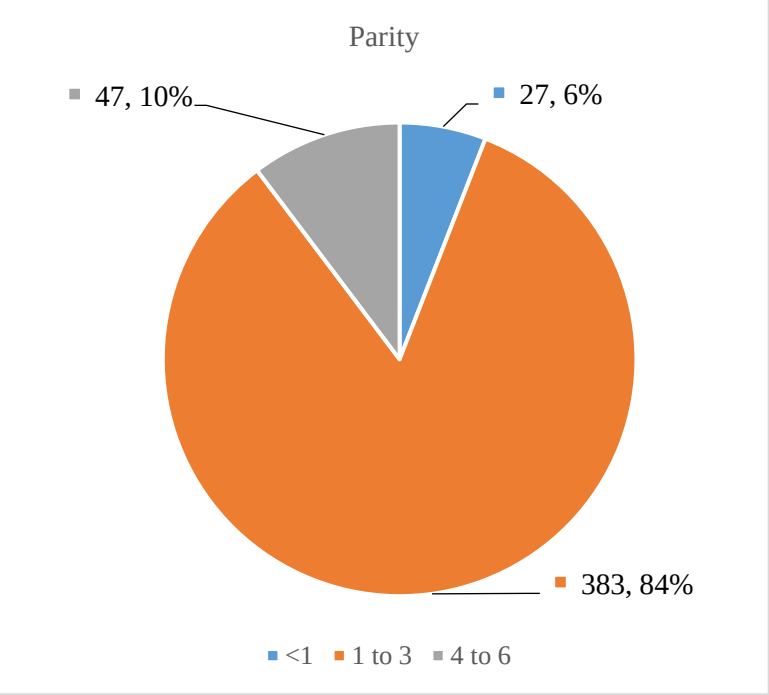


Figure 4.14: Parity from obstetrics history

- 90% females having parity. Others are shown in (Fig 4.14).

#### 4.3.10.3 Living

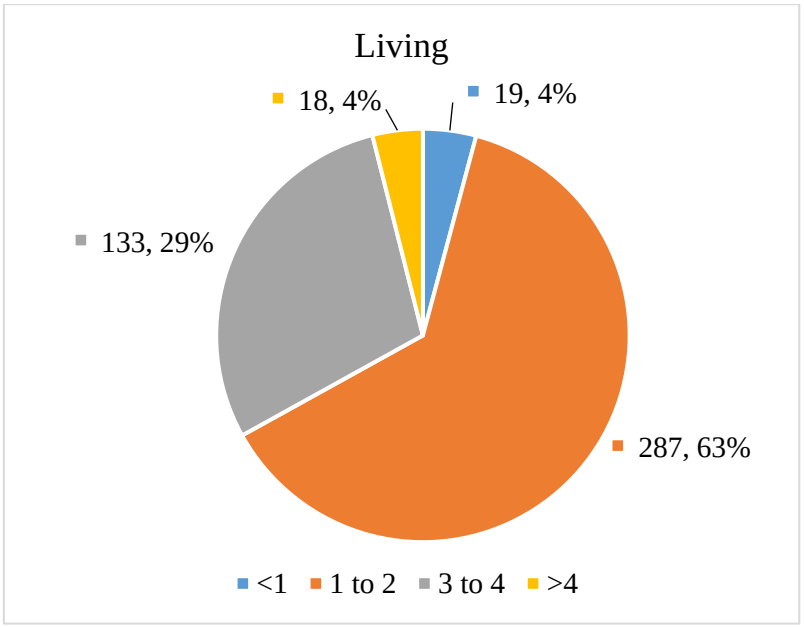


Figure 4.15: Living from obstetrics history

- 63% females had1or 2 child and both are living. 29% female have 3 or 4 children and 4% have more than four children. (Figure 4.15)

#### 4.3.10.4 Abortion

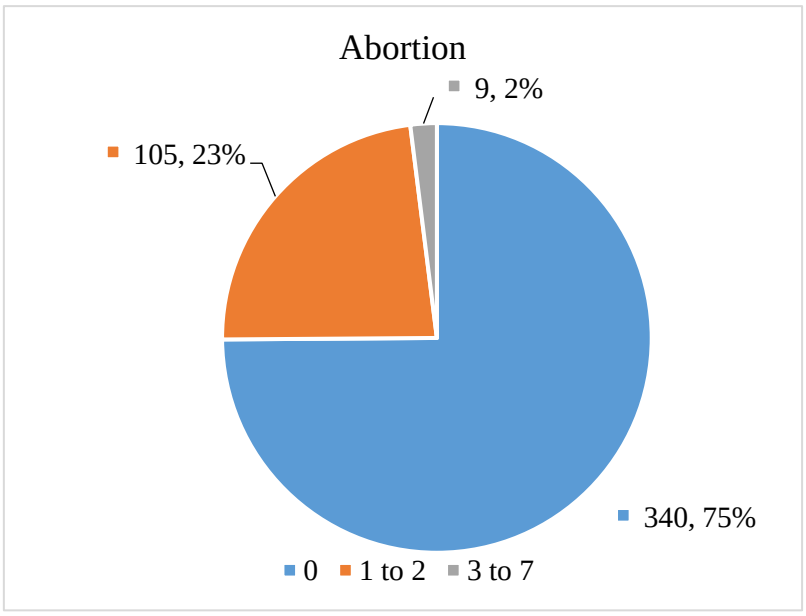


Figure 4.16: Abortion from obstetrics history

- 75% females had not any history of abortion, 23% women had one history of abortion and 2% female had history of more than one abortion. (Figure 4.16)

#### 4.3.10.5 Tubal Ligation

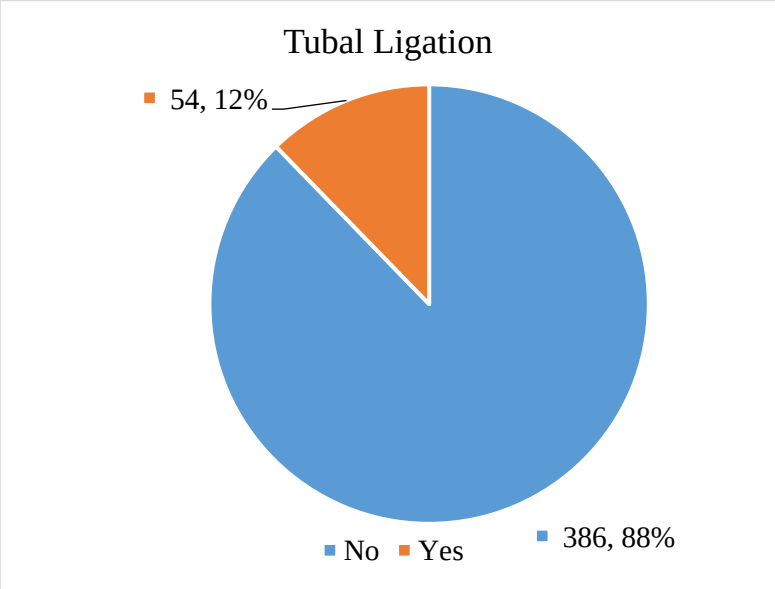


Figure 4.17: Tubal ligation from obstetrics history

- 88% female does not have any tubal ligation and only 12% have tubal ligation (Figure 4.17)

### 4.3.11 Clinical History

- We did not get sufficient data for past and personal history, family history, and medication history. Thus, it was excluded from the study for this section.
- 98% women were not taking any contraceptives whereas only 2% women were taking contraceptives.

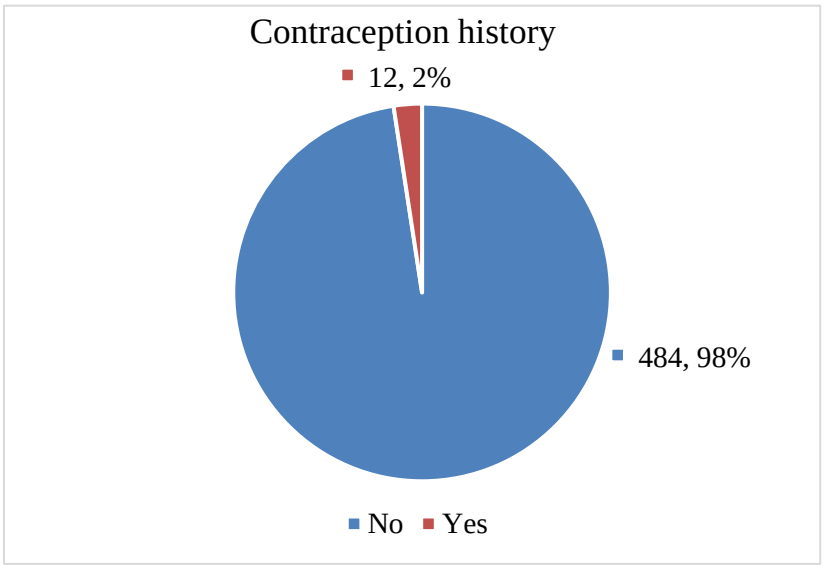


Figure 4.18: Contraception history of female

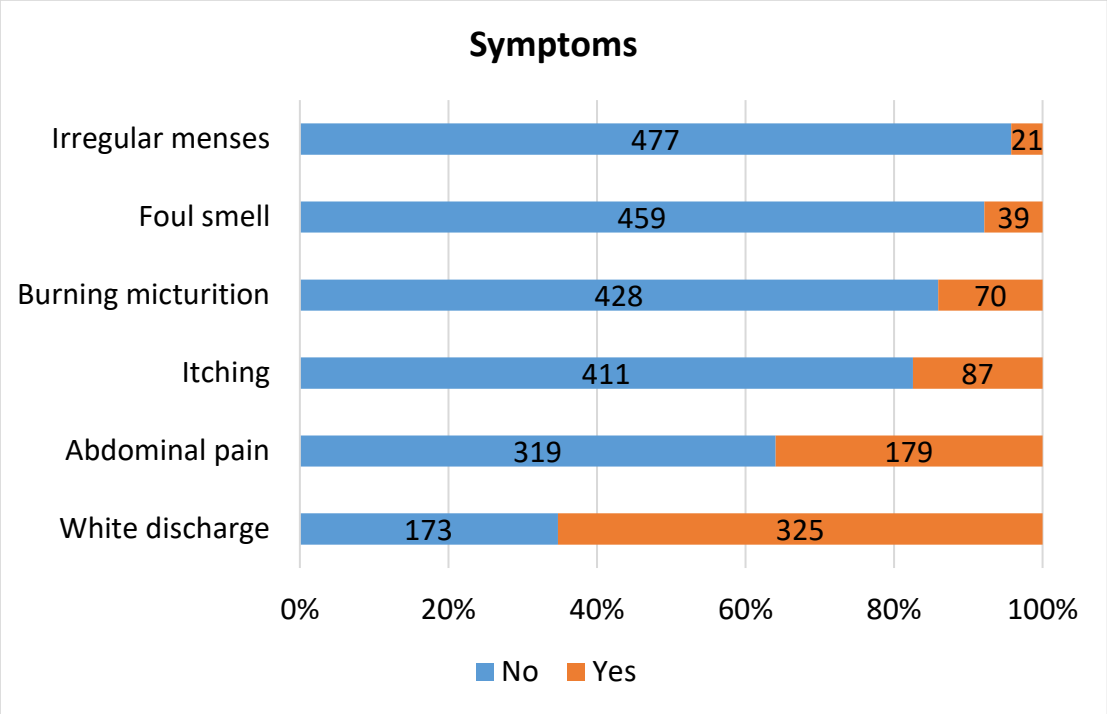


Figure 4.19: Common symptoms observed in cervical cancer screening

### 4.3.12 Sociodemography of menopausal women

- Data shows that 108 women were having menopausal symptoms which were given in the tables and figures below.

| Personal Data | Age | Frequency (n=108) | Percentage (n=108) |
|---|---|---|---|
| Age at a time of screening | 25-35 | 11 | 10.19 |
| | 36-45 | 60 | 55.56 |
| | 46-55 | 18 | 16.67 |
| | 56-65 | 11 | 10.19 |
| | >66 | 8 | 7.41 |
| Age of marriage | <18 | 7 | 6.48 |
| | 18 | 46 | 42.59 |
| | >18 | 50 | 46.30 |
| | not known | 5 | 4.63 |
| Active married life | <10 | 3 | 2.78 |
| | 11-20 | 18 | 16.67 |
| | 21-30 | 43 | 39.81 |
| | 31-40 | 32 | 29.63 |

| | | | |
|---|---|---|---|
| | >40 | 12 | 11.11 |
| Urban/Rural | Rural | 8 | 7.41 |
| | Urban | 100 | 92.59 |
| Community | Hindu | 94 | 87.04 |
| | Muslim | 14 | 12.96 |

Table 4.6: Personal data of patient having Menopause

- Highest menopausal age between 36-45 was 60. Most common age of marriage was more than 18 years. Active sexual life of women was seen in between 21-30 years was highest. In geographic region most of them from urban side of Saurashtra region. In Community most of them were from Hindu community.

| **Menstrual history** | **Age** | **Frequency(n=108)** | **Percentage** |
|---|---|---|---|
| Menarche | 12 | 1 | 0.93 |
| | 13 | 65 | 60.19 |
| | 14 | 14 | 12.96 |
| | 15 | 8 | 7.41 |
| | 16 | 4 | 3.70 |
| | 17 | 1 | 0.93 |
| Menopause | not known | 11 | 10.19 |
| | Physiological | 92 | 85.19 |
| | Surgical | 9 | 8.33 |
| | not known | 3 | 2.78 |

Table 4.7: Menstrual history of Menopausal women

- Most common age of menarche was13. Most menopause was physiological.

| **Labour History** | **Count** | **Frequency (n=104)** | **Percentage** |
|---|---|---|---|
| Mode of delivery | FTLSCS | 3 | 2.88 |

| | | | |
|---|---|---|---|
| | FTCS | 22 | 21.15 |
| | FTND/FTCS | 1 | 0.96 |
| | FTVD | 71 | 68.27 |
| | FTVD/FTCS | 2 | 1.92 |
| | FTVD/FTLSCS | 1 | 0.96 |
| Place of delivery | Hospital | 78 | 75.00 |
| | Home | 18 | 17.31 |
| | not known | 23 | 22.12 |

Table 4.8: Labour history of menopausal women

- Most common mode of delivery was FTVD. Most of women's place of delivery was hospital.

| **Obstetric History** | **Count** | **Frequency (n=104)** | **Percentage** |
|---|---|---|---|
| Gravidity | <1 | 2 | 1.92 |
| | 1-4 | 81 | 77.88 |
| | 5-8 | 20 | 19.23 |
| | 8-12 | 1 | 0.96 |
| | not known | 0 | 0.00 |
| Parity | <1 | 2 | 1.92 |
| | 1-4 | 89 | 85.58 |
| | 5-8 | 11 | 10.58 |
| | 8-12 | 0 | 0.00 |
| | not known | 2 | 1.92 |
| Living | <1 | 0 | 0.00 |
| | 1-4 | 88 | 84.62 |
| | 5-8 | 0 | 0.00 |
| | 8-12 | 0 | 0.00 |
| | not known | 0 | 0.00 |
| Abortion | <1 | 64 | 61.54 |
| | 1-4 | 16 | 15.38 |

| | | | |
|---|---|---|---|
| | 5-8 | 0 | 0.00 |
| | 8-12 | 0 | 0.00 |
| | not known | 10 | 9.62 |
| Tubal ligation | Yes | 14 | 13.46 |
| | No | 67 | 64.42 |
| | not known | 23 | 22.12 |

Table 4.9: Obstetric history of menopausal women

- Highest gravidity was between from5-8. Highest Parity was between 1-4. Most of was between1-4 in Living category. Most of women aborted 1-4 times in their lifetime. 67 was the frequency of tubal ligation out of 108.

| **Clinical history** | **Frequency** | **Percentage** |
|---|---|---|
| Contraception (n=108) | 3 | 2.8 |
| Medication (n=107) | 15 | 14 |
| Past (n=108) | 4 | 3.7 |
| Personal (n=107) | 6 | 5.6 |
| Family (n=108) | 2 | 1.9 |

Table 4.10: Clinical History of menopausal woman

- There is no connection of taking contraception with menopause because out of 108 women just 3 had contraceptives. There was no relation between medication and menopausal situation. There was no past history related to cancer or menopausal abnormalities. No bodies were having personal history of like cancerous symptoms or menopausal symptoms before besides 86 women. Only 2 have family history of both at a same time Cancer and Menopause.

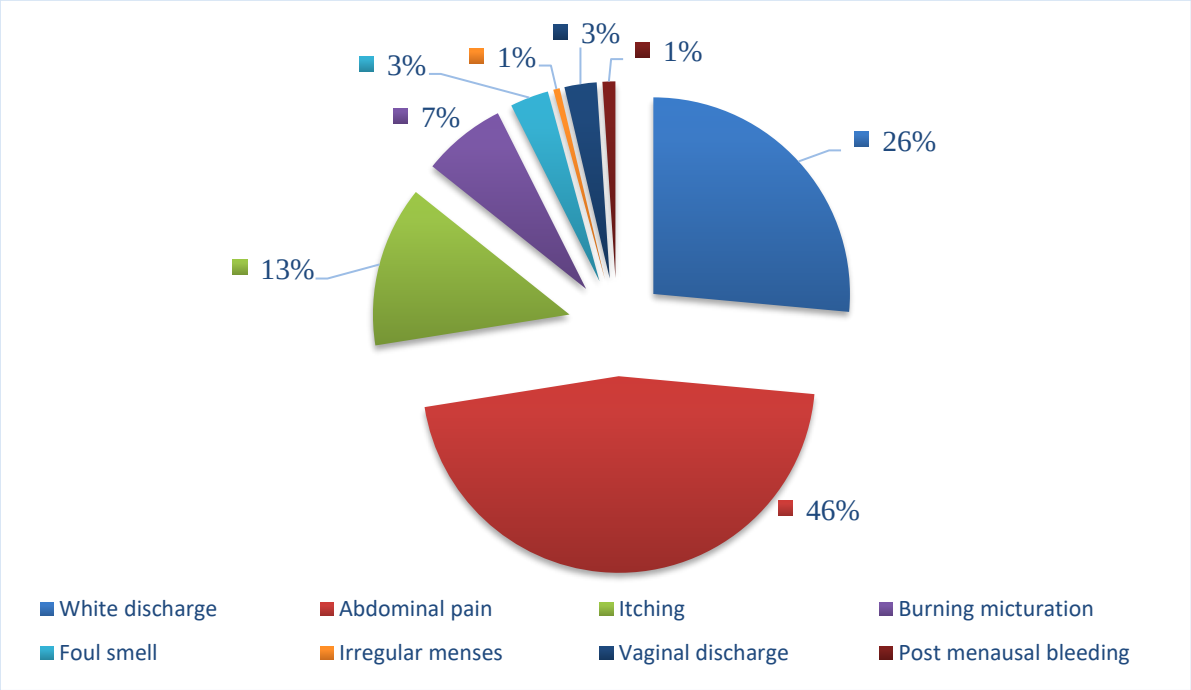


Figure 4.20: Symptoms observed in menopausal women

- Most common symptoms were Abdominal pain, white discharge, itching and burning micturiation

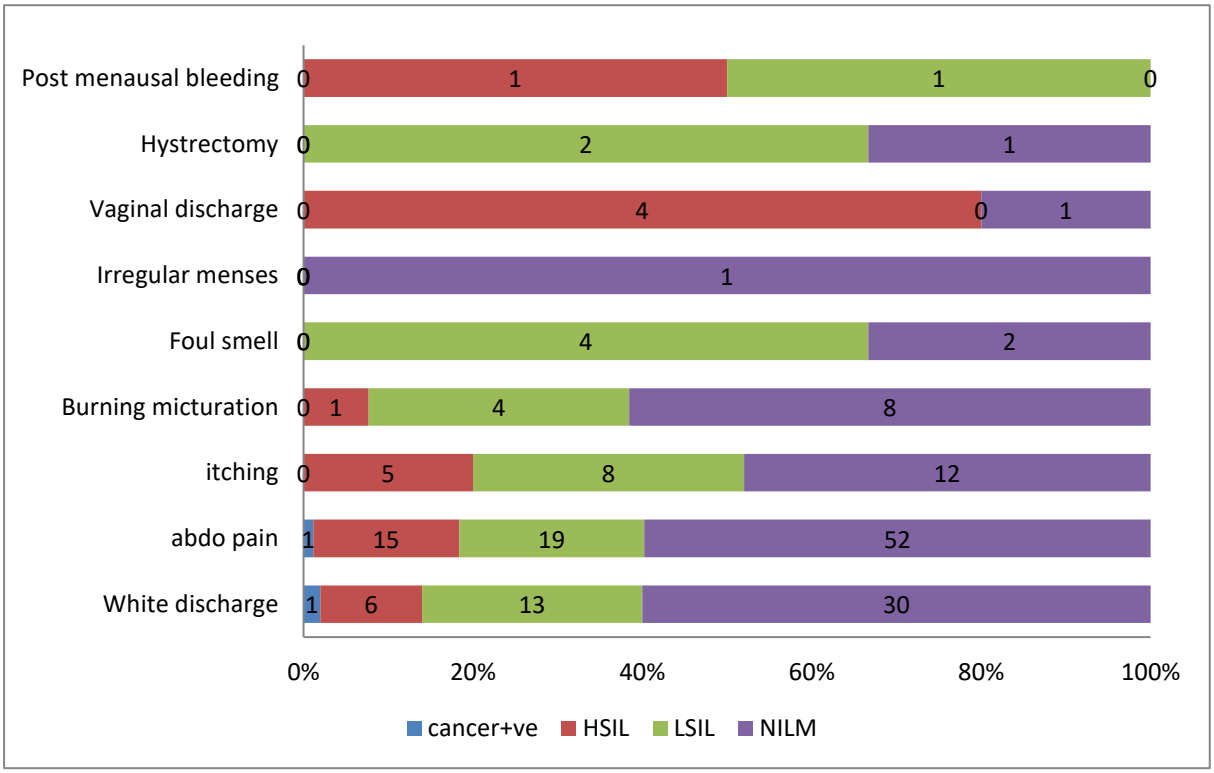


Figure 4.21: Symptoms v/s PAP results

- Pre-menstrual bleeding was most common symptom in comparison with PAP.

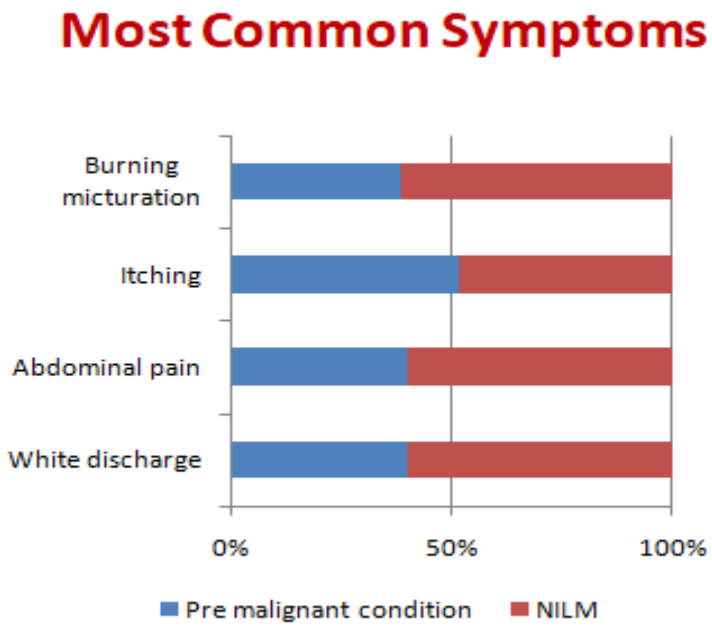


Figure 4.22: Most common symptoms in menopause

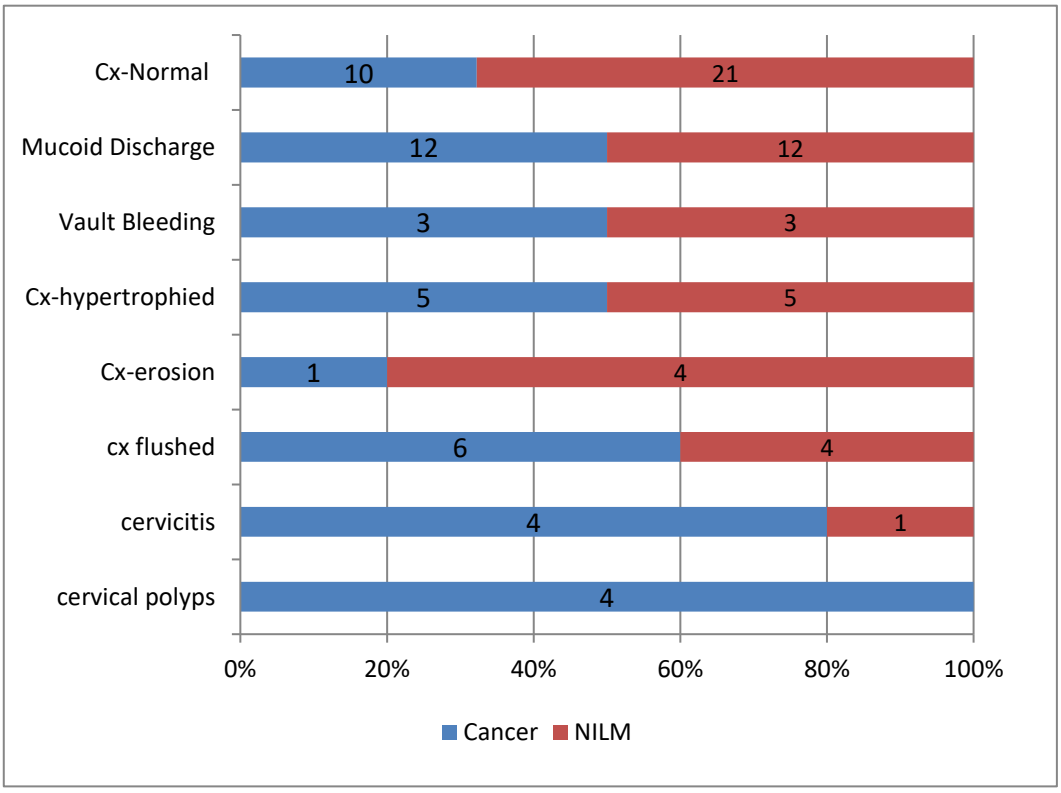


Figure 4.23: Pelvic Per Speculum Examination in menopausal women

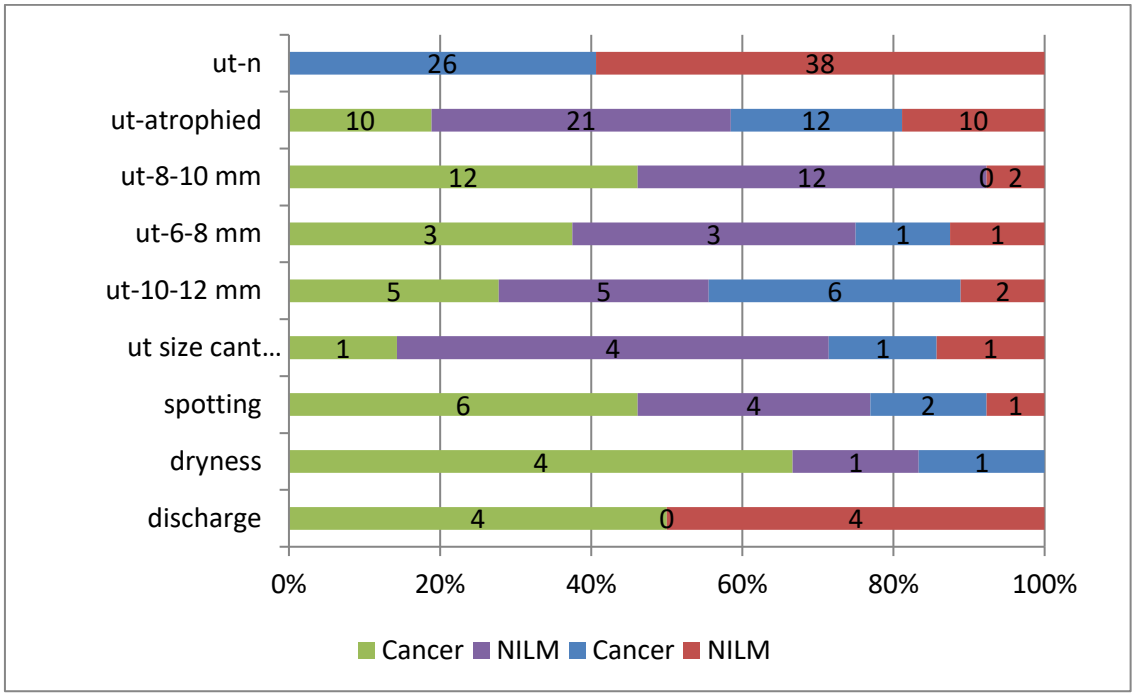


Figure 4.24: Pelvic Per Vaginal Examination in menopausal women

## 4.4 Visual Inspection

### 4.4.1 Pelvic Per Speculum (P/s) examination

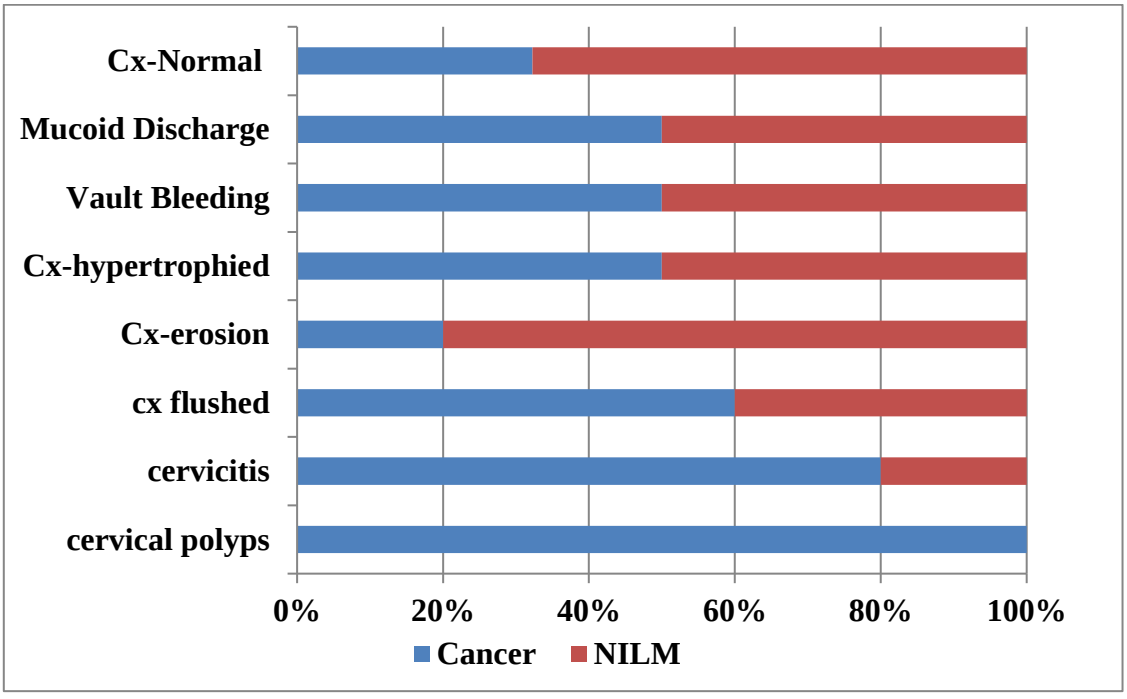


Figure 4.25: Showing pelvic per speculum examination

- Cervical polyps are observed in cancer observed in cancer patients only.

### 4.4.2 Pelvic Per Vaginal (P/v) examination

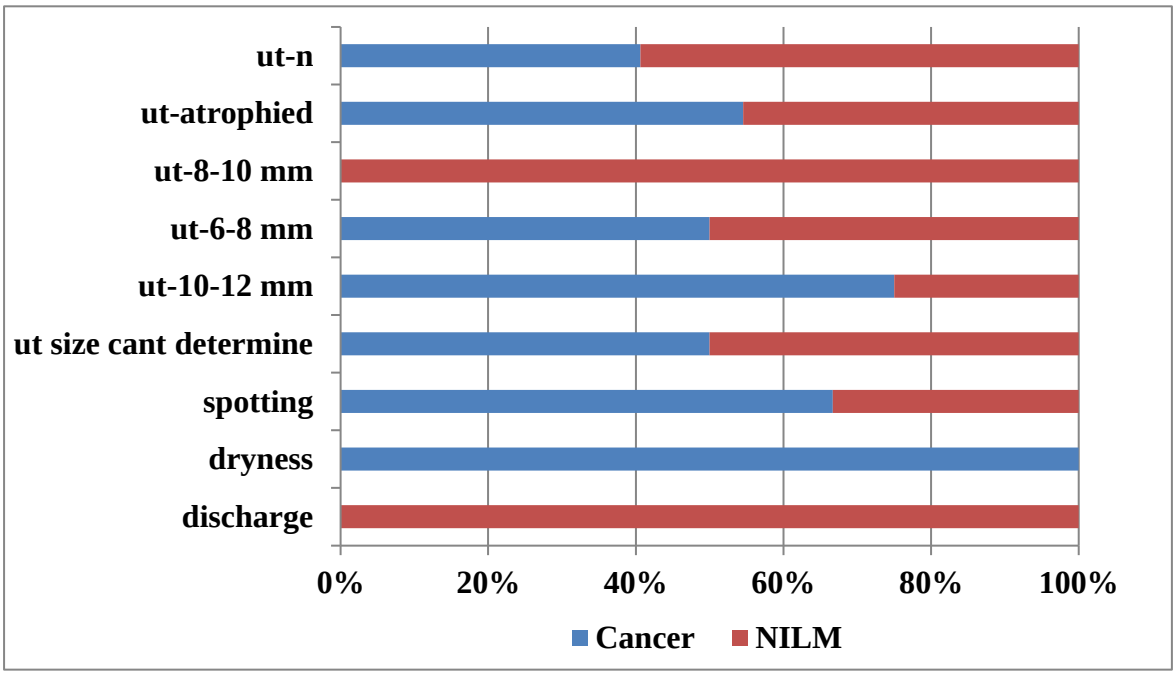


Figure 4.26: Showing pelvic per vaginal examination

- Dryness was most observed in pelvic per vaginal examination.

## 4.5 PAP and AgNOR staining results

### 4.5.1 PAP staining results

- Table shows data of PAP staining results obtained from cytopathology department.

| Grades | PAP staining |
|---|---|
| Cancer Positive | 3 (1%) |
| HSIL | 38 (8%) |
| LSIL | 66 (13%) |
| NILM | 391 (78%) |

Table 4.11: PAP staining results

### 4.5.2 AgNOR staining results

- We utilized the NOR counts of the silver-stained cervical scrap smears for a better understanding of the premalignant lesions and their transitions into low and high cervical intraepithelial lesions, before they got transformed into cervical cancer. So, NOR ultimately behaved as a tumor proliferative marker in our study.

AGNOR staining of cancer patients with different stages

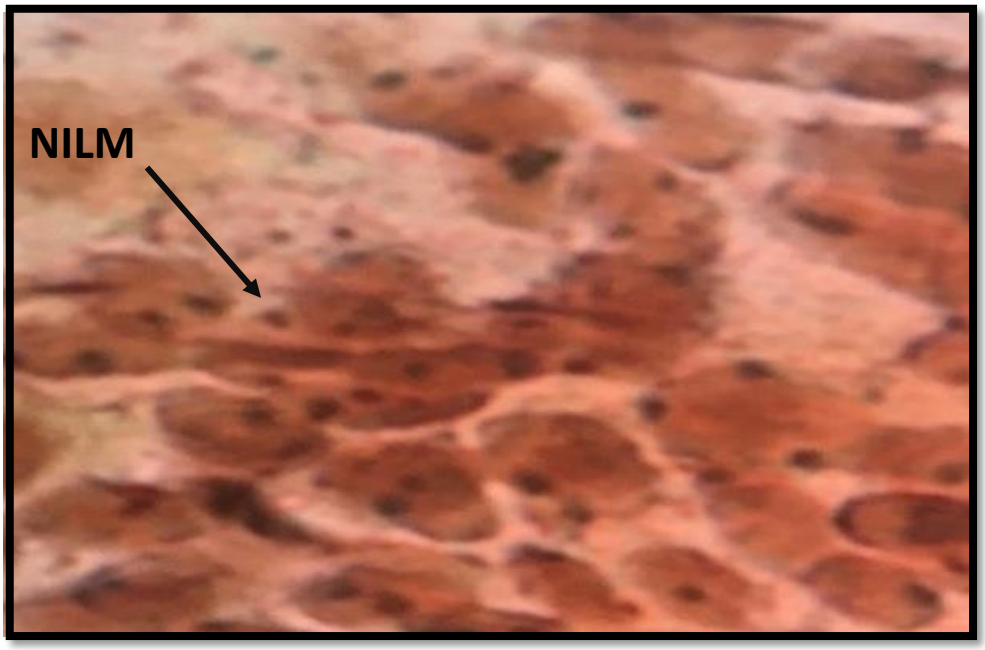


Figure 4.27: Normal cervix stained with silver stain showing predominantly 1 Dot (NOR)/cell (X1000)

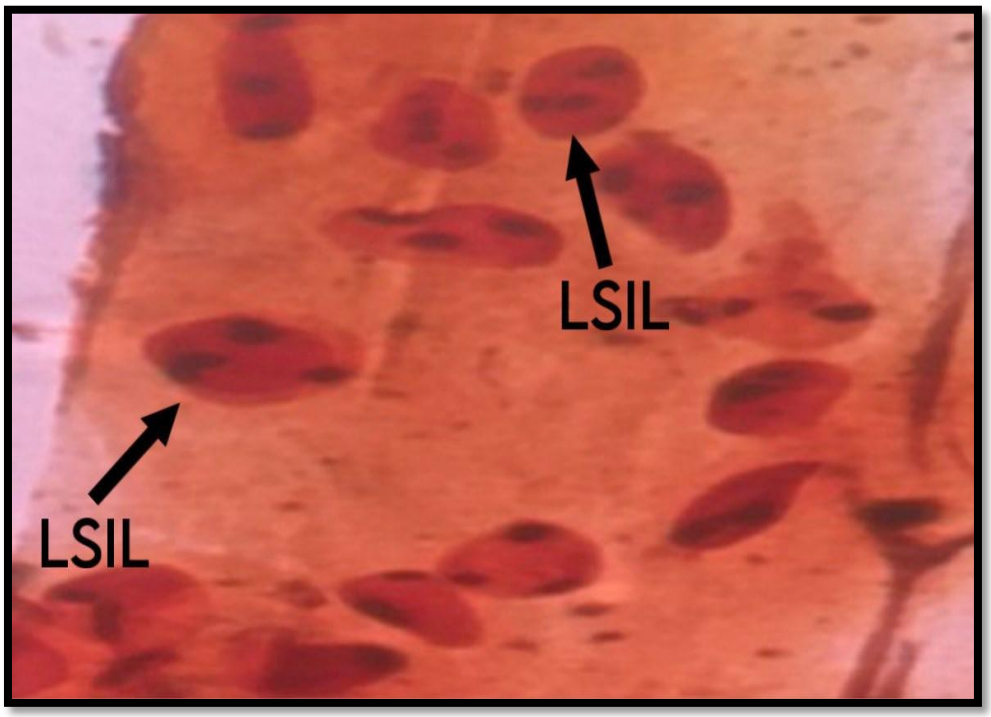


Figure 4.28: Low Squamous Intraepithelial Lesion (LSIL) Stained with Silver Stain Showing 2- 3 Dots (NOR)/ Cell (X1000)

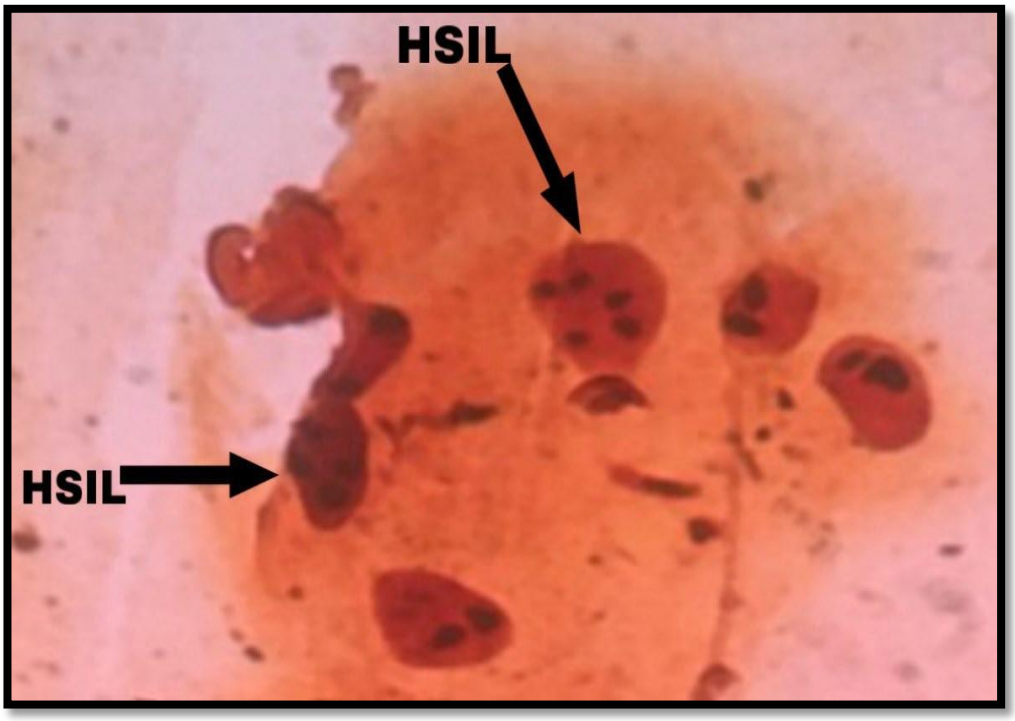


Figure 4.29: High Squamous Intraepithelial Lesion (HSIL)Stained with Silver Stain Showing 3-4 Dots (NOR)/Cell (X1000)

| Grades | PAP staining | AgNOR staining (%) |
|---|---|---|
| Cancer Positive | 3 (1%) | 3 (1%) |
| HSIL | 38 (8%) | 42 (8%) |
| LSIL | 66 (13%) | 55 (13%) |
| NILM | 391 (78%) | 398 (78%) |

Table 4.12: Grade wise comparison between AgNOR and PAP

### 4.5.3 Statistical analysis for comparison

- Specificity and sensitivity of Pap and AgNOR parameters among normal cervix, LSIL, HSIL, and cancer-positive were calculated using Microsoft Excel. Here we had taken NILM and LSIL as negative whereas HSIL and Cancer positive as positive for calculating sensitivity and specificity. Table 4.11 shows sensitivity and specificity of AgNOR against Pap.

| Statistic | Value | 95% CI |
|---|---|---|
| Sensitivity | 93.20% | 86.50% to 97.22% |
| Specificity | 100.00% | 99.06% to 100.00% |
| Negative Likelihood Ratio | 0.07 | 0.03 to 0.14 |
| Disease prevalence * | 18.00% | |
| Positive Predictive Value * | 100.00% | 0.00% to 0.00% |
| Negative Predictive Value * | 98.53% | 97.04% to 99.28% |
| Accuracy * | 98.78% | 97.36% to 99.55% |

*These values are dependent on disease prevalence.

Table 4.13: Comparison of PAP Staining and AgNOR staining

## 4.6 p53 and Ki67 staining results

- Ki-67 is a relatively positive sign of nuclear proliferation. It is also an important reference for predicting the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. Its expression can reflect the biological behavior of tumor cells.
- The p53 gene is an important tumor suppressor gene located in human chromosome 17p13.1. Its encoding product p53 protein plays an important role in cell division and differentiation. The normal expression of p53 protein can induce apoptosis and cause cell cycle block. p53 protein mutations can lead to cell transformation and over-proliferation and tumor behavior.
- The relationship between the percentage of cells with p53 expression i.e. its grading and histological diagnosis, the difference of p53 protein expression between CIN III and SCC was statistically insignificant (‘p’ value >0.05), showed positive association between the invasion of cervical lesions and expression of p53.

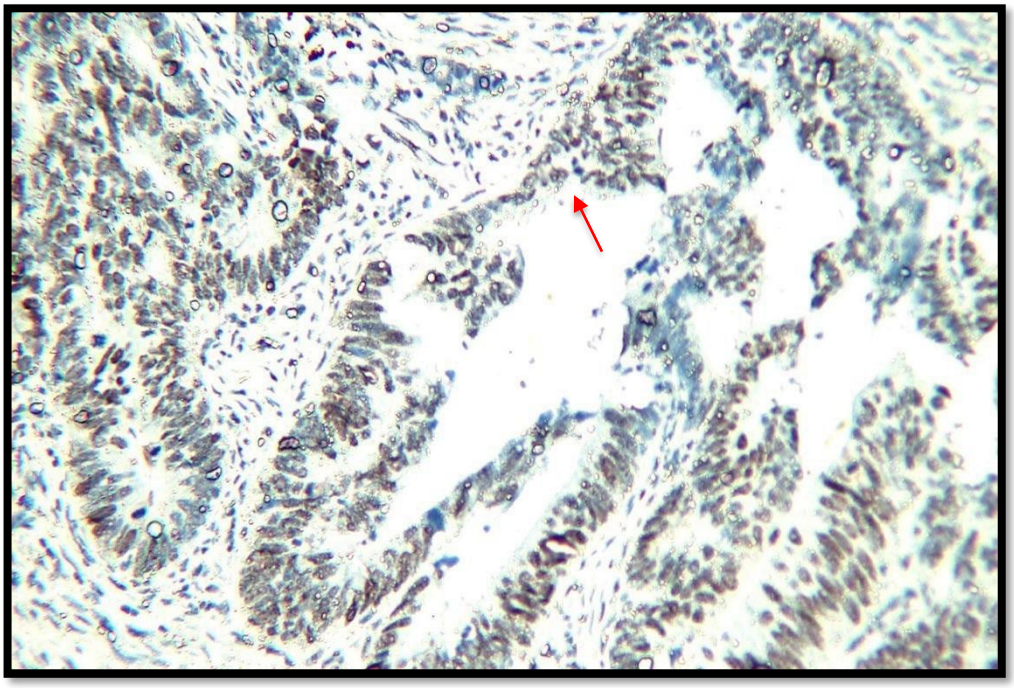

Figure 4.30: p53 showing positivity in HSIL smear (X400)

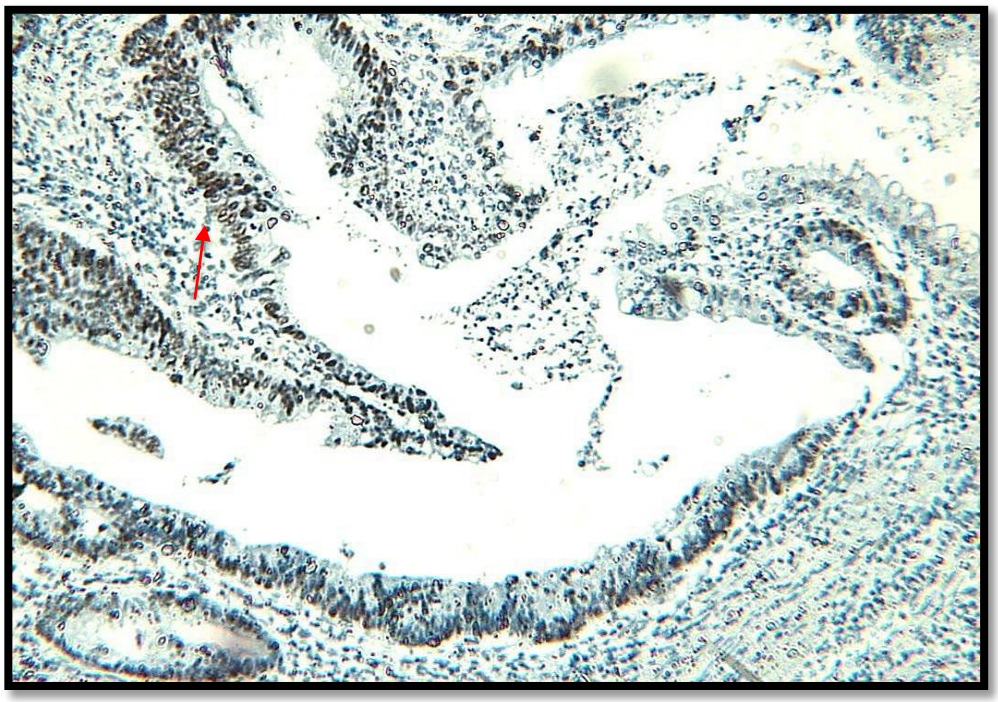

Figure 4.31: p53 showing positivity in malignant cervical neoplasm (X400)

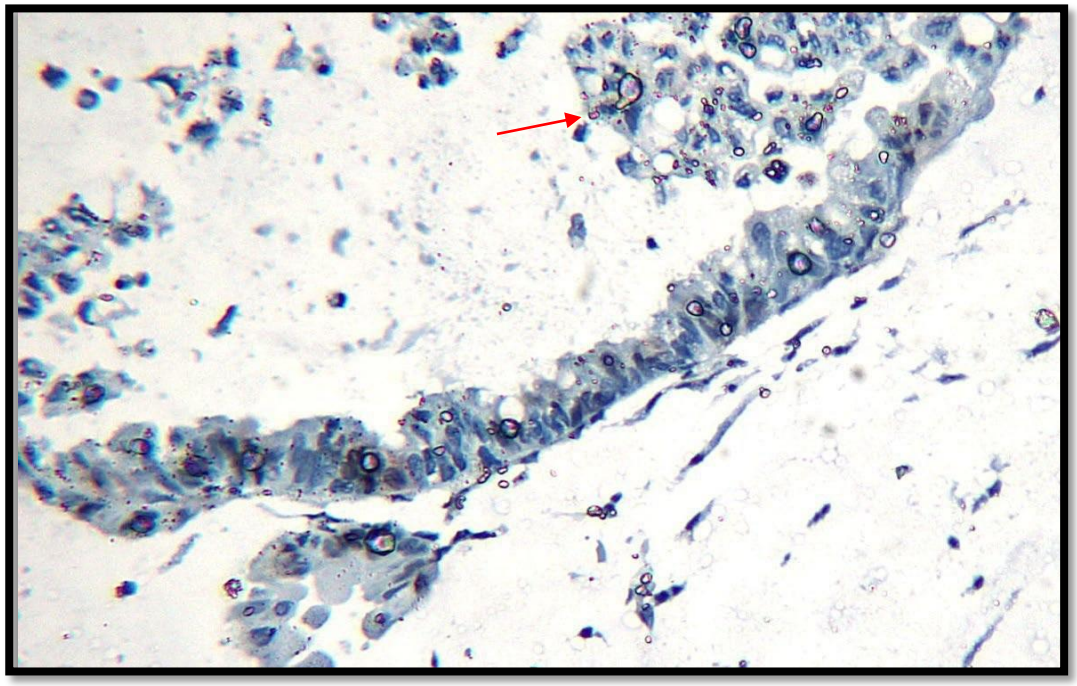

Figure 4.32: Ki-67 showing positivity in HSIL smear (X400)

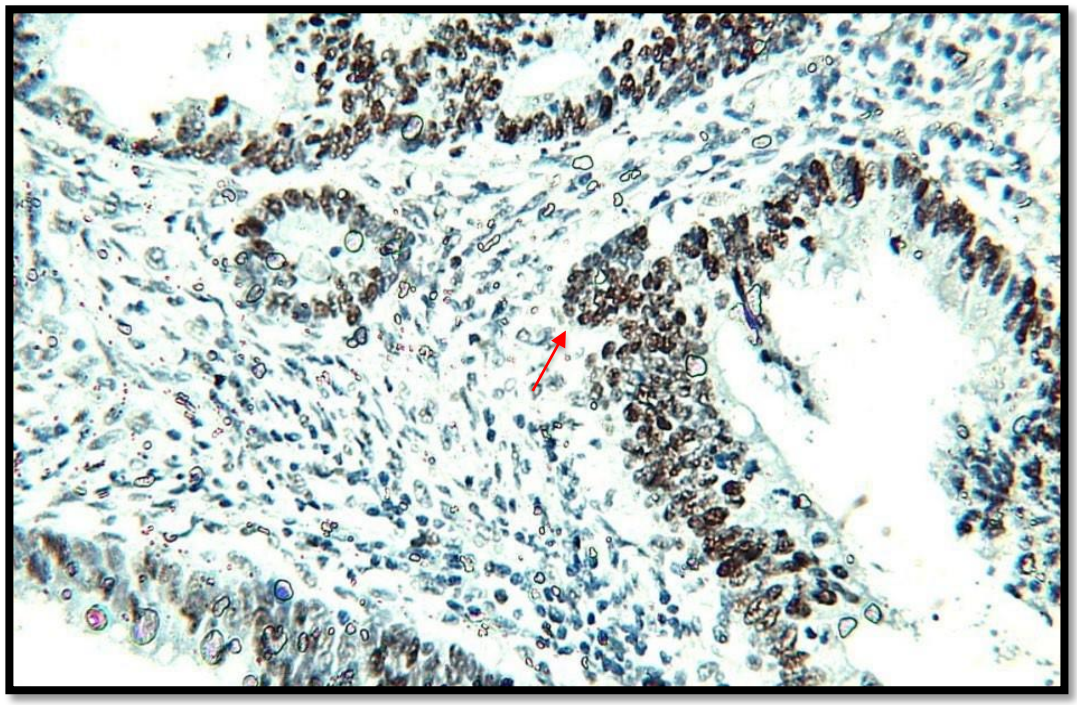

Figure 4.33: Ki-67 showing positivity in malignant cervical neoplasm (X400)

- When the intensity of staining is evaluated Grade-III or intense expression of p53 protein was observed in 21/31 (67.74%) of malignant cases, while only 5/19 (26.32%) of CIN III cases were immunoreactive. The difference

in the intensity of p53 protein immune-expression was statistically significant ('p' value <0.001 on log linear analysis).

| **Grades** | **AgNOR staining (%)** | **P53/Ki67 (%)** |
|---|---|---|
| Cancer Positive | 3 (1%) | 3 (2.9%) |
| HSIL | 42 (8%) | 38 (36.5%) |
| LSIL | 55 (11%) | 63 (60.6%) |
| NILM | 398 (80%) | - |

Table 4.14: Comparison between AgNOR and p53/ki67

### 4.6.1 Statistical analysis for comparison

- Specificity and sensitivity of ki-67/p53 and AgNOR parameters among normal cervix, LSIL, HSIL, and cancer-positive were calculated using Microsoft Excel. Here we had taken NILM and LSIL as negative whereas HSIL and Cancer positive as positive for calculating sensitivity and specificity. Table 4.13 shows sensitivity and specificity of AgNOR against ki-67/p53.

| **Statistic** | **Value** | **95% CI** |
|---|---|---|
| Sensitivity | 100.00% | 91.40% to 100.00% |
| Specificity | 100.00% | 94.31% to 100.00% |
| Positive Likelihood Ratio | | |
| Negative Likelihood Ratio | 0 | |
| Disease prevalence * | 18.00% | |
| Positive Predictive Value * | 100.00% | 91.40% to 100.00% |
| Negative Predictive Value * | 100.00% | 94.31% to 100.00% |
| Accuracy * | 100.00% | 96.52% to 100.00% |

*These values are dependent on disease prevalence.

Table 4.15: Sensitivity and Specificity of AgNOR considering ki-67/p53 as gold standard

## 4.7 Gel Electrophoresis of PCR product


Figure 4.34: Gel picture of amplification of beta-globin gene

- Using a 100 bp ladder, an amplified gene product was seen, with a DNA band visible between 200 and 300 bp where Lane 1: 100 bp DNA ladder. Lane 3 to 12: Cervical cancers smear samples. Lane 6 and 11: show the presence of the beta globin gene

## 4.8 HPV typing by RT PCR in cancer patients

- Figure 4.35 shows that the curve crossed the threshold line prior to completion of PCR cycles and thus it is positive for Type 16.

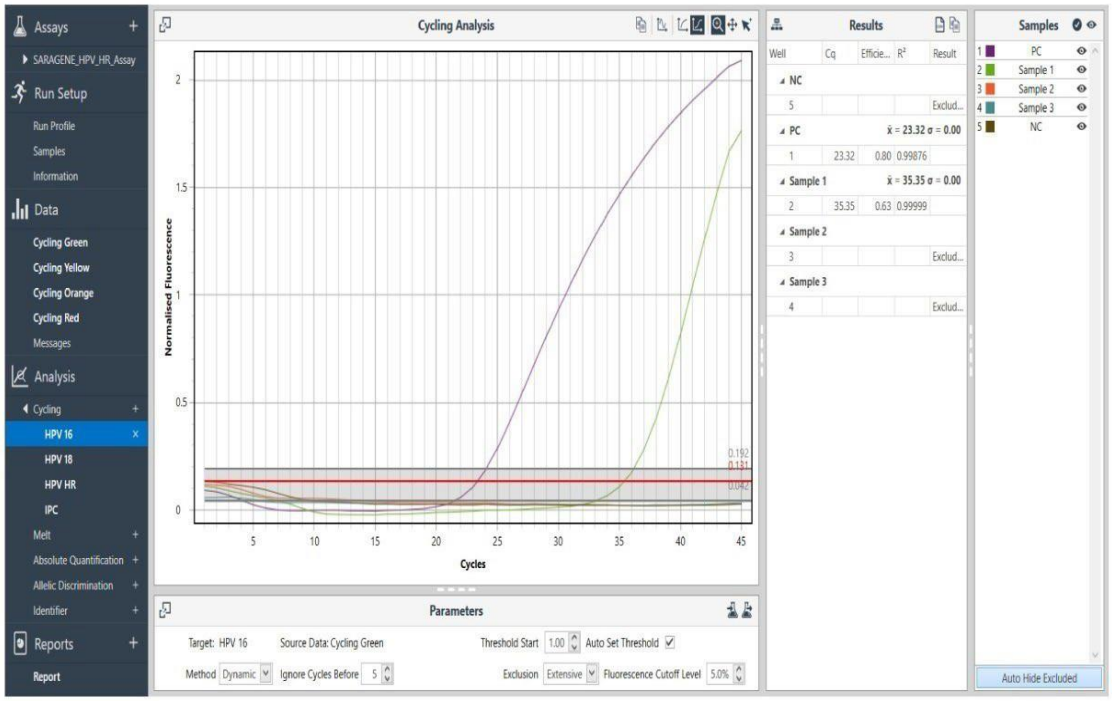


Figure 4.35. showing cancer patients samples positive for HPV 16 by Typing

- Figure 4.35 shows that the curve didn't cross the threshold line prior to completion of PCR cycles it remained below the threshold and thus it is negative for Type 18.

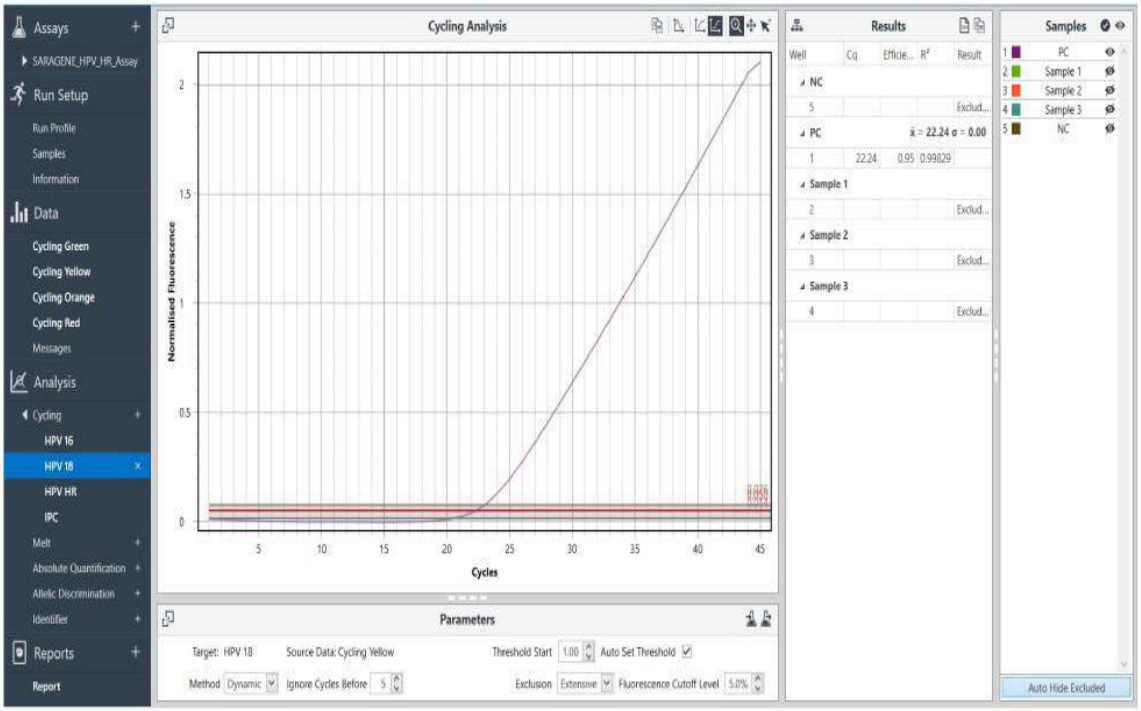


Figure 4.36: showing cancer patients samples negative for HPV 18 by Typing

Conventional PCR was used to detect HPV using beta-globin gene primers.

- Out of 3 samples 2 sample results of HPV-DNA typing showing type 16 and type 18 of Human Papilloma virus.

| **Grades** | **PAP staining (%)** | **AgNOR staining (%)** | **P53/Ki67 (%)** | **HPV PCR** | **HPV typing** |
|---|---|---|---|---|---|
| Cancer Positive | 3 (1%) | 3 (1%) | 3 (7.4%) | 3 | 2 (Type 16 & 18) |
| HSIL | 38 (8%) | 42 (8%) | 38 (92.6%) | 1 | - |
| LSIL | 66 (13%) | 55 (11%) | - | - | - |
| NILM | 391 (78%) | 398 (80%) | - | - | - |

Table 4.16: Cumulative results of different diagnostic methods

- P value is less than 0.05, which suggests that it is statistically highly significant and that means these groups are dependent and share relationship within and between groups. Table 4.17 shows the details of Anova test results.

| | | **Sum of squares** | **Degree of freedom** | **Mean square** | **F value** | **Significance level** |
|---|---|---|---|---|---|---|
| **HPV by PCR** | Between Groups | 191.327 | 3 | 63.776 | 5775.937 | < 0.05 |
| | Within Groups | 5.455 | 494 | 0.011 | | |
| | Total | 196.781 | 497 | | | |
| **AgNOR** | Between Groups | 195.152 | 3 | 65.051 | 2958.043 | < 0.05 |
| | Within Groups | 10.864 | 494 | 0.022 | | |
| | Total | 206.016 | 497 | | | |
| **ki67/p53** | Between Groups | 190.433 | 3 | 63.478 | 2930.657 | < 0.05 |
| | Within Groups | 10.7 | 494 | 0.022 | | |
| | Total | 201.133 | 497 | | | |

Table 4.17: ANOVA test results

# Discussion

## 4.9 Sociodemography and Cervical Cancer

According to GLOBOCAN 2020, cancer of the cervix uteri is the second most common cause of death in India, with a mortality rate of 9.1% and the third most prevalent cancer with an incidence rate of 18.3% (123,907 cases). The 5-year prevalence rate across all ages was 42.82 per 1 lakh people, whereas the age-standardized incidence rate per 100,000 people was 18 ("About cervical cancer in India," 2020).

The simplicity and low cost of the quantitative analysis that relies on counting each silver- stained NOR under an optical microscope are its main benefits (Gajewska, Kwiecień, Rutkowska, Rzepecki, & Sułek, 2022).

In our study, patients age ranges from 18 to 86 years, where most patients fall between 26 to 45 years. A study from Reichheld et al. found similar data in their study (Reichheld, Mukherjee, Rahman, David, & Pricilla, 2020). 49.4% of patients were married below the age of 18 years, whereas Reichheld et al. found 33.1%. Most of the patients were from urban areas, i.e., 96.4%.

More than 90% belong to the Hindu community. Reichheld et al. found that the study shows no children in 9.3% of the surveyed sample, whereas our study shows 5.9% (Reichheld et al., 2020). It is not clear how aging relates to the development of cancer. Nonetheless, it is worth mentioning that certain types of cancer occur more frequently in certain age groups (Dix & Cohen, 1999).

Analysis of data from this study revealed that the peak age of occurrence of cervical cancer was from 50-69 years, with a frequency of 57%. This finding closely aligns with the figures reported by Gustafsson et al.(Gustafsson et al., 1995). The researcher conducted a study and found that the peak value was between 50 and 70 years old. Additionally, Koulibaly et al. also conducted a similar study and reported similar findings (Koulibaly et al., 1997).

Dysplasia development has always been influenced by socioeconomic status, leading to an epidemiological impact. Chronic cervicitis, dysplasia, and invasive malignancies are more prevalent in the low-income group. Severe dysplasia accounts for 75% of cases, while invasive tumours account for 100% of cases in this group (Vuković, Bjegović, & Vuković, 2008). Vaidya's study sheds light on

the significant impact of low socioeconomic status on the development of dyskaryosis. According to his findings, individuals from low-income backgrounds are more prone to CIN II and CIN I, accounting for 50% and 80% of cases, respectively. The study highlights the factors that explain the correlation between low socioeconomic status and cervical cancer: poor personal hygiene, unsanitary housing conditions, unstable marital relationships, and early initiation of sexual activity. These findings underscore the need for a comprehensive approach to address the issue and improve the health outcomes of vulnerable populations (Bhattacharyya, Nath, & Deka, 2015).

Only 7.1% of the women aged 25 to 65 had ever had a cervical cancer screening (Reichheld et al., 2020). Women of any age can acquire cervical cancer, but it often does so between the ages of 35 and 55, with the peak age of occurrence varying depending on the community (J. B. Prasad & Dhar, 2018). In our study, cervical cancer patients range from 35 to 75. Similar findings have been reported by Arbyn and colleagues, who found that middle-aged women were primarily impacted by cervical cancer, particularly in underdeveloped nations (Marc Arbyn et al., 2020). This advanced age denotes a relative lack of knowledge and accessibility to comprehensive and equitable cervical cancer screening services in our nation. According to a study, age significantly affects the time and frequency of cancer screening (Sawaya, Kulasingam, Denberg, & Qaseem, 2015). According to earlier reports, screening incidence varies greatly between states and between districts. The average prevalence of screening among women aged 30-49 was found to be 31.0% (29.7-32.4), which is higher than our study (Van Dyne et al., 2019). The duration of a marriage, specifically the amount of time a person engages in sexual activity, plays a distinct role in the development of cervical dysplasia and cancer. Our research found that women who were married for a period of 5 to 20 years experienced dysplasia at a rate of 25-75%. Approximately 50% of participants were diagnosed with squamous cell carcinoma (Mekonnen & Mittiku, 2023).

We suspect multiple factors may affect women's ability and desire to participate in screening. The majority of women were willing to be screened for cervical cancer and knew of locations for screening, but most of the women did not know about the screening tests. Screening is a preventative service, which is not a priority for

asymptomatic and low-income people who are struggling with more acute day-to-day problems (Mehrotra et al., 2018).

Most of the women in our survey are housewives who rely on their husbands for financial support. The decision to pay for screening is generally made by their spouses, which adds another barrier to access for women seeking essential preventive care. If the financial barrier were removed, women might be more inclined to request screening (Suchitra et al., 2018). In general, women are generally not screened due to a fundamental lack of knowledge about prevention, time and financial constraints, and an overwhelming dearth of information regarding cervical cancer.

Previous research has shown that lifestyle and dietary choices are crucial for cervical cancer etiopathology and clinical outcome (Sharma et al., 2017). Although multiparous women were identified with cervical cancer at an earlier stage, but this link was not statistically significant. But it is generally known that multiparous women have an increased risk of developing cervical cancer (Shrivastav et al., 2021). A retrospective investigation carried out in a tertiary care hospital in Mangalore revealed similar results as well (Nilima, Puranik, Shreenidhi, & Rai, 2020). A retrospective investigation by Srivastava et al. revealed similar findings as well (Srivastava et al., 2018).

Aside from permanent sterilization, patients utilizing IUCD and OCP had a higher incidence of chronic cervicitis. 28.6% of those with chronic cervicitis were not using any treatment (Kaneshiro & Aeby, 2010). Among patients with dysplasia, 25% of those with moderate dysplasia used IUCD. However, 25% of those with mild dysplasia and 50% of those with severe dysplasia did not use any form of contraception. Additionally, 50% of patients with light and severe dysplasia were permanently sterilized. Among patients with squamous cell carcinoma, 50% were permanently sterilized or did not utilize any form of contraception (Averbach et al., 2018). One study found that women who use hormonal contraceptives have a higher risk of developing cervical dysplasia (Anastasiou et al., 2022).

Another research revealed that women who have given birth multiple times (multiparous women) are more likely to develop dysplasia and aggressive carcinomas. Among patients with severe dysplasia, 25% had a Parity 2 classification, 50% had a Parity 3 classification, and 25% had a Parity 4 or higher

classification. In patients with invasive cancer, 50% had a P2 classification, 35% had a Parity 3 classification, and 30% had a Parity 4 or higher classification (Ashing-Giwa et al., 2004). Shalini et al. conducted a study which found that patients diagnosed with invasive cancer had an average parity of 4.2 (Shalini, Amita, Neera, & investigation, 1998). Kushtagi and Fernandez's research showed that the frequency of CIN was significantly higher in women who had given birth more than twice (Kushtagi P, 2002). Similarly, Vaidhya's study revealed that women with a parity greater than 4 had more cases of CIN (A, 2003). According to Adadevoh et al. and Becker et al., this may be attributed to hormonal and dietary changes that occur during pregnancy, immunosuppression during pregnancy, and cervical damage during vaginal delivery (Adadevoh & Forkouh, 1993; Rudlowski et al., 2003).

Patients with chronic cervicitis most commonly exhibit erosion (81%) and moderate dysplasia (75%) during clinical examination. In cases of significant dysplasia, the cervix is severely damaged and tends to bleed upon touch. For patients with invasive squamous cell and adeno carcinoma, a friable growth or ulcer is often the first symptom observed (D. Prasad et al., 2021). In our study, white discharge, was followed by abdominal pain, itching, urning micturiation and foul smell as the most prevalent clinical symptoms associated with the development of cervical cancer. Other research has observed similar results as well with different weightage (*Health Technology Assessment of Strategies for Cervical Cancer Screening in India*; Shrivastav et al., 2021).

Excessive vaginal discharge is a common complaint among women with chronic cervicitis, dysplasia, or metaplasia. According to a study by Vaidya et al., such discharge can also lead to the development of CIN. The study found that 24% of the women surveyed reported experiencing vaginal discharge (A, 2003).

## 4.10 Argyrophilic Nucleolar Organizer Regions and Cervical Cancer

It was observed that the AgNOR dots were single larger and compact in the normal cervix. They appeared small and loosely arranged in the dysplastic and malignant lesions of the cervix (Shukla et al., 2013). The unique Nucleolar Organizer Regions (NORs) are emphasized on staining with the silver stains and can truly help in specifically recognizing the cervical neo-plastic status, preventing a misdiagnosis

with the currently used AgNOR stain. The frequency of the severity of the lesions is correlated with an increase in the NOR counts (Khieu M). According to Eagan et al., the average AgNOR count increased consistently while the average AgNOR size decreased from CIN I to CIN II. A statistically significant difference in AgNOR counts between CIN I, CIN II, and CIN III was also demonstrated earlier (Egan, Freeth, & Crocker, 1990; Goyal et al., 2012). The factors that can alter the AgNOR counts (diminished AgNOR results) are - improper collection of the smears, not collecting them from the proper sites/ lesions, inadequate cellularity, inter and intra observer errors, etc. Besides this, the cervical smear results are directly related to the staining reagent. The staining reagent should be freshly prepared (Khieu M).

Ploton et al. discovered through their 1986 research that the reaction between solutions A and B is endothermic, and as a result, the rate of reaction would rise with rising temperatures. The staining period was shorter as the staining temperature was raised (Ploton et al., 1986). Continually increasing with the grade of the histological lesions is AgNOR polymorphism. This can be used to predict how squamous cell carcinoma will develop in the future. Similar results from the current investigation, which focused on precancerous and cancerous cervix lesions, demonstrated the AgNOR's value as a cellular activity marker and as a malignancy marker (Alarcón-Romero LC, 2009).

The cervical smears were the subject of the current study. AgNOR counts are a reliable indicator of cell proliferation. As the severity of the cervical lesions (cervical lesions- inflammatory, LSIL, HSIL, and cervical cancer) increases, so do its counts. The PAP-based cervical smear diagnosis is greatly aided by assessing a NOR count since it provides a significant concept about the type of intraepithelial lesion or cancer in the cervical region (Mohanty & Padhy, 2020).

Alarcon-Romero et al (2009) found that a high number of large kidney-shaped and clustered AgNORs in premalignant and malignant conditions of the cervix was associated with malignant transformation probably due to HPV infection. He also suggested that combined study of viral parameters and AgNOR polymorphism may be useful as a prognostic factor for estimating the progression of premalignant lesions to squamous cell carcinoma (Alarcón- Romero LC, 2009).

In a study, it was found that there were more cases of pre-invasive lesions (HSIL and LSIL) in females with unhealthy cervix (24 out of 69) as compared to those

with healthy cervix (7 out of 36). This difference was statistically significant ($p < 0.05$). Additionally, when punch biopsy specimens were examined, it was observed that majority of the women (44 out of 52) were diagnosed with CIN2+ lesions, of which two-thirds were suffering from CaCx. These findings suggest that in this region, patients tend to visit gynecologists late in the process of developing cancer (Jayant, Rao, Nene, & Dale, 1995). A similar study conducted elsewhere in India also concluded that women in India tend to ignore the signs and symptoms of cancer until it's too late, and are often reluctant to attend community-based cancer screening programs (Senapati, Nayak, Kar, & Dwibedi, 2017).

During conventional Pap smear screening, the presence of koilocytes is still considered as an indicator of HPV infection. However, the presence of koilocytes does not distinguish between low-risk and high-risk types of HPV. It is important to note that not all HPV infections show morphological expression (Baak et al., 2006). Additionally, the diagnostic accuracy of cervical preinvasive lesions is compromised due to low sensitivity, specificity, and predictive value of cytologic and histopathologic diagnoses, mainly because of inter-observer variability (Krawczyk et al., 2008).

When analysing the age distribution of different cervical lesions, it was observed that adenocarcinoma had the highest mean age of 61 years, while SCC had a mean age of 49.7 years. Although the age range for LSIL and HSIL was quite broad, 21-66 and 36-60 years, respectively, the mean age was 42.2 years for LSIL and 46 years for HSIL. This observation emphasizes the importance of screening for cervical cancer in all females entering their 4th decade of life (Vahabi & Lofters, 2016).

In a retrospective study of 146 cases of cervical cancer in Nigeria, 79% of cases were diagnosed in advanced stages. The peak age incidence was between 40 and 70 years with a mean age of

54.5 years (Onwudiegwu, Makinde, Ezechi, Adeyemi, & Gynaecology, 1999). A comparable investigation was carried out to evaluate astrocytic lesions of the brain [19], to differentiate between various histopathological grades of Oral Epithelial Dysplasia [20], to examine bone marrow [21], and to diagnose biliary tract carcinoma (Nanashima et al., 2002) too.

Our study indicates the usefulness of AgNOR count and PCR protocol for detection of HPVs from smear samples of cervix. Alternative screening test of AgNOR is simple and can be used as proliferative marker. Individual AgNOR formations were distinguished based on the size of their surface areas and thus we believe that this type of analysis is rapid, precise, and impartial compare to conventional techniques. We recommend developing software for measuring size of the NOR to provide precise diagnostic results and treatment plan.

## 4.11 Menopause and Cervical Cancer

In this study, mean age of menopause was 52.2 years. we found that 56% of women were in the age of less than 35 years at the time of screening which seems uncommon in compare to other studies. Possible reason may be surgical menopause. Though women screened for cervical cancer belongs to this age group were 45% (n=498) and it may be due to their awareness and education. Misconceptions concerned with older age and menopause may reduce women's perceived susceptibility to cervical cancer and exert a negative effect on their screening behavior. Most of the women underwent cervical screening as part of a medical checkup, which proves that organized screening programs helps to maximize participation of the desired population. Additionally, if health professionals refer the patient may boost trust in the screening programs (Tacken et al., 2007). Mathew et al found that less than 1% women belongs to less than 35 years of age (Mathew et al., 2019). Neha EL et al found 36.5% cases of carcinoma cervix which belonged to the age group of 50 to 60, 42.3% cases belonged to age group of 60 to 70 (Neha E. L., June 2017).

In this study, mean age of marriage was 20.01 years. we found only 6.5% women having marriage at the age of less than 18 years. Jissa V Thu et al study found 35.1% women having marriage at the age of less than 18 years (J. V. Thulaseedharan, Malila, N., Hakama, M., Esmy, P. O., Cheriyan, M., Swaminathan, R., ... Sankaranarayanan, R., June 2012).

In case of geographical entities, we found 93% women from urban area as compare to rural place. Whereas Mathew et al found 59% women from rural and 49% from urban area (Mathew et al., 2019).

In case of community, our study found 13% women form Muslim community whereas 87% were Hindu. Whereas Mathew et al found 10% women form Muslim community whereas 71% were Hindu and 18% were Christian and other community were less than 1% (Mathew et al., 2019).

In this study, mean for menarche was 13.5. We found 60.2% women having menarche at the age of 13 years followed by 13% at the age of 14 years. Our study found younger women which were aged less than 35 years. The incidence rates of early menopause in women raise a serious public health concern (5%, early menopause; 1%, premature menopause) (Faubion, Kuhle, Shuster, & Rocca, 2015). Larger numbers of women are at risk of cervical cancer due to early menopause (J. M. Kim, Yang, Lee, & Jee, 2021). In this study we found that 68.3% deliveries were FTVD, whereas FTCS were 21.2%. In our study, 75% deliveries were done at hospital. Home delivery percentages were very low i.e.,17.3%. In this study, we found 77.8% gravidity in range of 1-4. In Mekonen et al study they found 62.1% having less or equal to 2 (Mekonen et al., 2021). In this study, we found that 85.58% parity in range of 1-4. Whereas Mekonen et al found 71.2% in less or equal to 2 (Mekonen et al., 2021). In this study, we found 71.29% of patients with no abortion whereas Mekonen et al found 87.9% (Mekonen et al., 2021).

In this study we found 2.8% women using contraceptive measures whereas a study by Sreejata Raychaudhuri and Sukanta Mandal shows 61.5% women were using contraceptive measures (Raychaudhuri, April 2012). In this study 14% women were found on different medication mostly due to diabetes and hypertension. Neha E. L. et al found that 5.7% taking medication related to diabetes and 13.4% were of hypertension (Neha E. L., June 2017).

Family history: In this study, we found 1.9% having family history whereas Neha E. L. et al found 9.5% patients having family history of cancer (Neha E. L., June 2017).

Identifying the complaints of menopausal women can support in the designing of training programs, increase the awareness and also ensures the good quality life (Hajesmaeel-Gohari, Shafiei, Ghasemi, & Bahaadinbeigy, 2021). In this study we found 1% postmenopausal bleeding whereas Mahadik et al found 4.69%. Few studies found 3.5% patients having complaints of post-menopausal bleeding were having cervical cancer (Ko, Tambouret, Wilbur, & Goodman, 2011; Mahadik JD,

2017; Sreedevi, Javed, & Dinesh, 2015). We found 3% vaginal discharge in menopausal women whereas Radu et al found 11% (Radu, Matos de Melo Fernandes, Khalfe, & Stordal, 2023). In this study we found 1% women with irregular menses whereas Radu et al found 22% (Radu et al., 2023). In this study we found 3% women with foul smell whereas Kumari et al found 31.25% (Kumari, Neelam, Yadav, & Sciences, 2017). In this study we found 7% women with burning micturition which is quite similar to Mahadik et al i.e. 7.38% (Mahadik JD, 2017). Chi square test was performed by us and result found was 1.96 and p value was 0.06 which states that there is no significant correlation. In this study we found 13% women having itching in perineal region whereas Mahadik et al found 2.01% (Mahadik JD, 2017). Chi square test was performed by us and result found was 0.95 and p value was 0.32 which states that there is no significant correlation. Women having complaint of abdominal pain in this study were 46% whereas in Mahadik et al, and Ko et al was 0.9% and 17.44% respectively (Ko et al., 2011; Mahadik JD, 2017). Chi square test was performed by us and result found was 1.96 and p value was 0.16 which states that there is no significant correlation. In this study we found 26% women complaining about white discharge whereas Mahadik et al found 9.89% (Mahadik JD, 2017). Chi square test was performed by us and result found was 0.46 and p value was 0.49 which states that there is no significant correlation.

Examination findings in Per speculum examination shows in this study we found 9.3% women having normal cervix whereas Mahadik et al found 50.16% (Mahadik JD, 2017). In this study we found 11.1% women having mucoid discharge whereas Mahadik et al found 0.16% (Mahadik JD, 2017). In this study we found 2.8% women having vault bleeding whereas Mahadik et al found 0.16% (Mahadik JD, 2017). In this study we found 4.6% women having Cervix hypertrophied whereas Mahadik et al found 1.67% (Mahadik JD, 2017). In this study we found 0.9% women having cervix erosion whereas Mahadik et al found 3.35% (Mahadik JD, 2017). In this study we found 5.6% women having cervix flushed whereas Mahadik et al found 3.2% (Mahadik JD, 2017).

In case of per vaginal examination in this study we didn't found any such case but Mahadik et al found 21.64% with such observation (Mahadik JD, 2017). In this study we found 1.9% women having spotting whereas Mahadik et al found 0.33%

(Mahadik JD, 2017). In this study we didn't found any such case but Mahadik et al found 9.89% women having discharge (Mahadik JD, 2017). In this study we found 0.9% women observed with dryness whereas Mahadik et al found 0.16% (Mahadik JD, 2017).

Burning micturition, itching, white discharge, and abdominal pain were the most common symptoms among menopausal women. In the Pelvic per speculum, Mucoid discharge and in the Pelvic per vaginal uterus, atrophied was the most common complaint. It was observed that there was no significant difference between symptoms of menopause and pre-cancerous condition. Thus, we found menopause is a high-risk factor for HPV infection, and its symptoms must be evaluated precisely. Mis conceptualized behaviour of menopausal women also affects the cancer screening program and early diagnosis. It is suggested that menopausal women should screen themselves at least annually.

## 4.12 Human papilloma Virus and Cervical Cancer

In present study beta globin gene was present in 3 out of 4 samples. HPV was considered as the most important risk factor in the pathogenesis of Carcinoma Cervix after the demonstration of integrated HPV-16 and -18 genome in the biopsy specimens and cell lines derived from HPV-positive Cervical cancer in the early 1980s (Boshart et al., 1984).

The detection of HPV-16/-18 in samples from both healthy and unhealthy cervix at comparable rates highlights the importance of HPV testing in all eligible females, regardless of the physical status of their cervix. HPV-16 positivity, which was associated with the majority of pre- invasive and invasive lesions, was significantly higher in females with unhealthy cervix ($p<0.05$), indicating a greater potential for malignancy. The increasing positivity of HPV-16/- 18 in low to high grade pre-invasive lesions, and its association with 1/4th of invasive lesions, such as CaCx, further substantiate the carcinogenic potential of these genotypes, particularly HPV-16. Previous studies have claimed that HPV-16 and -18 account for approximately 70% of CaCx cases [53].

Adenocarcinoma of the cervix has also been claimed to be associated with HPV, but the correlation is less pronounced and seems to be age dependent. HPV was

present in 89% of ADC in women younger than 40 years, whereas in women aged 60 years or older, HPV was observed in only 43% of cases (Bosch, 2011).

According to a report by the World Health Organization, the percentage of individuals in India with normal cytology who tested positive for HPV-16 and -18 was 4.7% and 1.3%, respectively. The variation in the percentage of HPV16/18 positivity across different studies may be due to differences in the techniques used to detect these viruses (Prakash et al., 2016). It was found that 25% of cases with reactive cellular changes tested positive for HPV-16/-18. The high positivity rate for HPV-16/-18 could be attributed to the increased sensitivity of the nested PCR protocol used. has the ability to detect even small amounts of target DNA, even in the presence of relatively high amounts of non-target human DNA that may inhibit the Taq polymerase enzyme during PCR (Prakash et al., 2014; Westra, 2014).

The importance of regular cytological screening of HR HPV associated NILM cases cannot be overstated. It was observed that HPV16/18 was present in both study groups, regardless of the physical status of the cervix as seen on clinical examination. Considering the significance of HR HPV testing, current US cervical cancer screening guidelines recommend prolonging the screening interval to a minimum of 3 years in women aged 30 or above with NILM cytology who test negative for HR HPV. Women who test positive for HR-HPV should be retested at 12 months using cervical cytology and HPV tests (Agorastos et al., 2015).

High-risk HPV testing has proven to be useful both in cervical cancer screening and management. The latest recommendations for cervical cancer screening, which were released in March 2012 by two separate groups, the USPSTF and a multidisciplinary partnership among the ACS, ASCCP, and ASCP, now suggest that combined cervical cytology and HPV testing is the preferred strategy for women aged 30 and above. These recommendations are also supported by the American College of Obstetricians and Gynecologists (ACOG) ("The American College of Obstetricians and Gynecologists cervical cancer screening," 2012).

In our study, we were able to identify the presence of HPV-16 and -18. Similarly, various studies conducted in other parts of India have also shown that genotype -16 and -18 are the most common in both pre-cancerous lesions and cancerous tissues of the cervix. These two genotypes were found to be associated with 68-88%

of cervical cancer tissue samples collected from different centres in Tamil Nadu and Maharashtra (P. Das et al., 2012; Revathidevi, Murugan, Nakaoka, Inoue, & Munirajan, 2021). In both the eastern city of Guwahati and the western city of Chandigarh in India, a high percentage of cervical samples tested positive for genotypes 16 and 18. These genotypes are commonly found in those regions (Aggarwal et al., 2012; B. Das et al., 1992).

HPV-16 and HPV-18 detection can offer useful insights for assessing risk and managing the clinical care of patients infected by these two genotypes with low grade lesions. It can also bean effective primary screening method for women aged 30 years or older (Tang et al., 2017; Q. Zhang et al., 2018).

In 3 cases where substitution occurred, mutations resulted in two types of L1 variants: A6665C and A6647C. The intratype variant A6665C is one of the most widely spread L1 variants and was detected in CaCx tissue samples from all participating centers, including Delhi, Thiruvananthapuram, Mumbai, Kolkata, Bangalore and Vellore (Pillai et al., 2009). This variant was also reported in China in 2011 (Ren et al., 2020). On the other hand, L1 variant A6647C, which was detected in one of the CaCx tissue samples subjected to sequencing in this study, has not been reported in India to date. This variant has been previously identified in Greece in HPV31, a closely related genotype of HPV16 (Pande et al., 2008). In a multicentric study conducted in India, intratype L1 variant C6240G at the genome level and H228D at the protein level were observed in 100% of the samples from all participating centers, except Delhi (Pillai et al., 2009).

The achievement of the goal requires supporting community-based screening programs with a proper referral system. Additionally, secondary and tertiary health centers need to be equipped to manage the cases detected by screening (Patro & Nongkynrih, 2007). Screening for CaCx is crucial for all eligible females regardless of cervix appearance on clinical examination.

A study carried out in India involving 142,701 women aged between 30-59 years, found that in a low resource setting, undergoing a single round of HPV testing can result in a considerable decrease in advanced cervical cancer cases and subsequent mortality (Sankaranarayanan et al., 2009).

The present study utilized a highly sensitive technique called nested PCR to detect the presence of HPV-16 and -18. This method has the potential to significantly

enhance the accuracy of screening programs for cervical cancer, while minimizing the risk of unnecessary treatment. By identifying the specific strains of HPV that are most commonly associated with cervical cancer, healthcare providers can better tailor their treatment plans to individual patients, resulting in more effective and personalized care.

## 4.13 p53/ki-67 and Cervical Cancer

There is significant variation in the expression of the p53 antigen in cervical cancer cells across different regions of the world. In one study, 61.7% of cervical tumor cells were found to be positive for p53 (Lopes et al., 2002). This value is similar to the 70.1% p53 positivity found in Malaysian women by Cheah et al (Garima, Pandey, Pandey, Saxena, & Patel, 2016). The study by Hunt et al. in Manchester found that 17.1% of 80 cases of cervical carcinomas were positive for p53, which differs from our results (Hunt, Hale, Buckley, & Hunt, 1996) whereas our study shows 39.4% positive for p53.

In a study conducted by Oka et al. in China, lower p53 values were reported (Oka, Nakano, & Arai, 1993). Similar studies were conducted by Kainz et al. in Australia (Kainz et al., 1995) and Busby-Earle et al. in Edinburgh (Busby-Earle, Steel, Williams, Cohen, & Bird, 1994), with positivity rates of 25.5%, 20.2%, and 14% respectively. Ngan et al. also studied the expression of p53 protein in 55 cases of cervical cancer in Hong Kong and found that only 3.6% of them exhibited the protein (Ngan, Stanley, Liu, & Ma, 1994). In a study in South African patients out of 50 cases of cervical cancer cases studied, 48 (86%) were positive for p53 protein (Abd El All, Rye, & Duvillard, 1999). Though most studies have not been able to establish a direct relationship between the level of p53 expression and cervical carcinoma, this study points out the possibility of a link between differential expression of p53 in cervical cancer cases.

This study supports the idea that mutations in the TP53 gene can play a significant role in the development of cervical cancer and can affect the prognosis. There is a higher mortality rate associated with this type of cancer in areas where the protein is expressed more. Apart from the usual cases of late presentation and lower treatment compliance in Africa, the higher level of p53 expression in cervical cancers from this part of the world may partly explain the higher mortality rates

observed in this region. Tumours that express higher levels of p53 tend to have higher mortality rates (Olivier, Hollstein, & Hainaut, 2010).

The tissue blocks stained with the Ki-67 antibody indicate that 59.7% of all cervical cancer cases tested positive for the Ki-67 antigen. This finding is consistent with other studies, which have found Ki-67 positivity rates of approximately 57.8% and 80.7% (H. Li et al., 2006; Munhoz et al., 2009).

Researchers found that 94.4% of Chinese women with cervical cancer had ki-67 positivity. (Tan et al., 2008). Cervical cancers typically have a uniform level of ki67 positivity, with most studies indicating over 50% positivity. Tissue blocks that tested positive for Ki67 were mostly collected in recent years, while older blocks showed lower expression of Ki67. This may be due to antigen loss in the older blocks. It is therefore recommended that more recent tissue blocks be used for Ki67 testing.

Although the relationship between p53 and Ki-67 expression and the various histological variants of cervical cancer was not statistically significant, the expression of p53 and Ki67 was higher in large cell nonkeratinizing and keratinizing squamous cell carcinoma than in adenocarcinomas. Similar observations were made in other studies, which also found differential expression of p53 in different histological variants (Hunt et al., 1996). This study contained many cases of squamous cell cervical carcinoma, which may explain the differential pattern of p53 and Ki-67 expression observed.

The study had some limitations that affected its findings. One of the main limitations was the inability to retrieve many tissues blocks of cervical cancer as they were not found. Additionally, some of the tissue blocks had insufficient tissue left for immunohistochemistry. A few blocks also did not contain representative histological sections appropriate for immunohistochemistry. It's important to note that the study was conducted in a hospital-based setting, meaning that not all cases of cervical cancer in Ile-Ife were included in the study because some patients with cervical cancer did not seek treatment at the hospital.

The expression of p53 was found in 61.7% of all cervical cancer cases studied, while Ki-67 was expressed in 59.7% of cases. Even though there was no statistically significant relationship found between the histological variants of cervical cancer and the staining of p53 and Ki-67, it was observed that a majority of the cases that

were positive for these antibodies were of the large cell non-keratinizing variant. Additionally, positive cases were found more frequently in recent tissue blocks. The study recommends conducting more research to establish the relationship between p53 and Ki-67 with cervical cancer, and performing immunohistochemistry tests, especially for Ki-67, on recent tissue blocks.